DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF FEW NOVEL DIAMINOPIMELATE DECARBOXYLASE INHIBITORS OF TUBERCULOSIS

A Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI - 600 032.

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTI- TUBERCULAR AGENTS TARGETING DIAMINOPIMELATE DECARBOXYLASE (LysA)"** submitted by the candidate bearing Register No.261415717 in partial fulfillment of the requirement for the award of the degree of MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY by The Tamilnadu Dr. M.G.R Medical University is a bonafide work done by him during the academic year 2015-2016 under my guidance at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-3.

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LIST OF ABBREVATIONS USED

ARG	Arginine
BCG	Bacillus Calmette Guerin
DAPDC	DiAminoPimelate DeCarboxylase
DFMO	Diluoromethylornithine
GPCR	G-protein-coupled receptors
HIS	Histidine
HPLC	High Performance Liquid Chromatography
HTS	High-Throughput Screening
INH	Isoniazid
iNOS	Nitric Oxide Synthase
IUPAC	International Union of Pure and Applied Chemistry
Lys	Lysine
MS	Mass Spectroscopy
MST	Micro Scale Thermophoresis
MTB	Mycobacterium tuberculosis
NMR	Nuclear Magnetic Resonance
ODC	Ornithine Decarboxylase

OECD	Organisation for Economical Co-operation and Development
PAS	Para Amino Salicylic acid
PDB	Protein Data Bank
PLP	Pyridoxal-5-Phosphate
PPD	Purified Protein Derivative
QSAR	Quantitative Strucural Activity Relationship
RNA	RiboNucleic Acid
RNIs	Reactive Nitrogen Intermediates
SAR	Structure acivity Relationship
SER	Serine
TB	Tuberculosis
TLC	Thin Layer Chromatography
TLR2	human toll-like receptor 2
PMN	Polymorphonuclear Leukocyte
LTBI	Latent Tuberculosis Infection
PE/PEE	Proline- Glutamate/ Proline –Proline- Glutamate
IR	Infra Red
NMR	Nuclear Magnetic Resonance

GC-MS	Gas Chromatography – Mass Spectrometry
LC-MS	Liquid Chromatography – Mass Spectrometry
1,4NAGT	1,4-N-Acetyl Glycosaminyl Transferase
D-3-PD	D-3-Phosphoglycerate Dehydrogenase
P-5-PO	Pyridoxamine-5-Phosphate Oxidase
DADAC	D-Alanyl D-Alanine Carboxypeptidase
XDR-TB	Extensively Drug Resistant- TB
ADME	Absorption, Distribution, Metabolism and Excretion
PSA	Polar Surface Area
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System
Log P	Partition Co-Efficient
MIC	Minimum Inhibitory Concentration
MABA	Micro Plate Alamar Blue Assay
TPSA	Total Polar Surface Area

CONTENTS

1. INTRODUCTION

TUBERCULOSIS- AN INSIGHT

Tuberculosis, MTB, or TB (short for *tubercle bacillus*) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people, who have an active TB infection, cough, sneeze, or otherwise transmit their saliva through the air. Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected.

Consumption, phthisis, scrofula, Pott's disease, and the White Plague are all terms used to refer to tuberculosis throughout history. It is generally accepted that the microorganism originated from other, more primitive organisms of the same genus Mycobacterium. Human bones from the Neolithic show presence of the bacteria, although the exact magnitude (incidence and prevalence) is not known before the 19th century.

The first references to tuberculosis in Asian civilization are found in the Vedas. The oldest of them (Rigveda, 1500 BC) calls the disease *yaksma*. The Atharvaveda calls it another name: *balasa*. It is in the Atharvaveda that the first description of scrofula is given. The *Sushruta Samhita*, written around 600 BC, recommends that the disease be treated with breast milk, various meats, alcohol and rest. The Yajurveda advises sufferers to move to higher altitudes.

Aretaeus was the first person to rigorously describe the symptoms of the disease in his text *De causis et signis diuturnorum morborum*:

"Voice hoarse; neck slightly bent, tender, not flexible, somewhat extended; fingers slender, but joints thick; of the bones alone the figure remains, for the fleshy parts are wasted; the nails of the fingers crooked, their pulps are shriveled and flat...Nose sharp, slender; cheeks prominent and red; eyes hollow, brilliant and glittering; swollen, pale or livid in countenance; the slender parts of the jaws rest on the teeth as, as if smiling; otherwise of cadaverous aspect..." **[1] [2]**

PATHOGENESIS OF TUBERCULOSIS

M. tuberculosis usually enters the alveolar passages of exposed humans in an aerosol droplet, where its first contact is thought be with resident macrophages, but it is also possible that bacteria can be initially ingested by alveolar epithelial type II pneumocytes. This cell type is found in greater numbers than macrophages in alveoli, and *M. tuberculosis* can infect and grow in these pneumocytes ex vivo.

In addition, dendritic cells play a very important role in the early stages of infection since they are much better antigen presenters than are macrophages and presumably play a key role in activating T cells with specific *M. tuberculosis* antigens. Since dendritic cells are migratory, unlike differentiated macrophages, they also may play an important role in dissemination of *M. tuberculosis.*

The bacteria are phagocytosed in a process that is initiated by bacterial contact with macrophage mannose and/or complement receptors. Surfactant protein A, a glycoprotein found on alveolar surfaces, can enhance the binding and uptake of *M. tuberculosis* by up regulating mannose receptor activity.

On the other hand, surfactant protein D, similarly located in alveolae, inhibits phagocytosis of *M.tuberculosis* by blocking mannosyl oligosaccharide residues on the bacterial cell surface, and it is proposed that this prevents *M.tuberculosis* interaction with mannose receptors on the macrophage cell surface. The human toll-like receptor 2 (TLR2) also plays a role in *M. tuberculosis* uptake.

On entry into a host macrophage, *M. tuberculosis* resides in an endocytic vacuoule called the phagosome. If the normal phagosomal maturation cycle occurs, i.e., phagosome-lysosome fusion, these bacteria can encounter a hostile environment that includes acid pH, reactive oxygen intermediates (ROI), lysosomal enzymes, and toxic peptides. The presence of RNIs in human macrophages and their potential role in disease has been the subject of controversy, but the alveolar macrophages of a majority of TB-infected patients exhibit iNOS activity. [1]

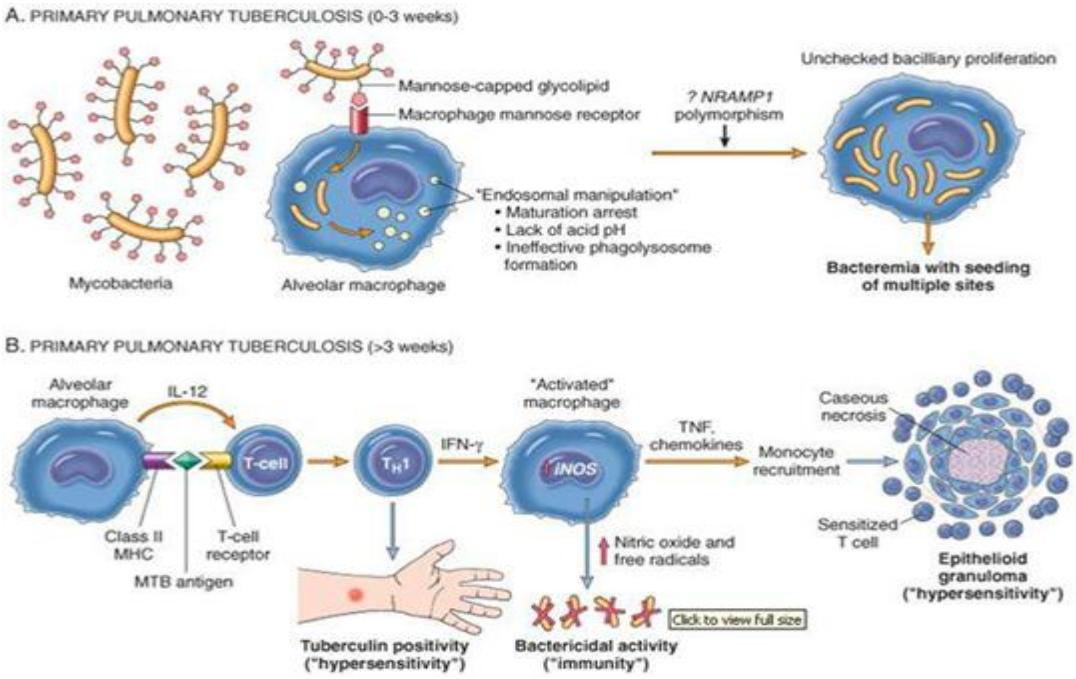


Fig 01 - Pathogenetic Pathway of tuberculosis infection

CELL WALL STRUCTURE

The cell wall structure of *Mycobacterium tuberculosis* deserves special attention because it is unique among prokaryotes, and it is a major determinant of virulence for the bacterium. The cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids. Over 60% of the mycobacterium cell wall is lipid.

The lipid fraction of MTB's cell wall consists of three major components, mycolic acids, cord factor, and waxD.

Mycolic acids are unique alpha branched lipids found in cell walls of *Mycobacterium* and *Corynebacterium*. They make up 50% of the dry weight of the mycobacterial cell envelope. Mycolic acids are strong hydrophobic molecules that form a lipid shell around the organism and affect permeability properties at the cell surface. Mycolic Acids are thought to be a significant determinant of virulence in MTB. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule.

Cord Factor is responsible for the serpentine cording mentioned above. Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strains of MTB.

WaxD in the cell envelope is the major component of Freund's complete adjuvant (CFA). [3]

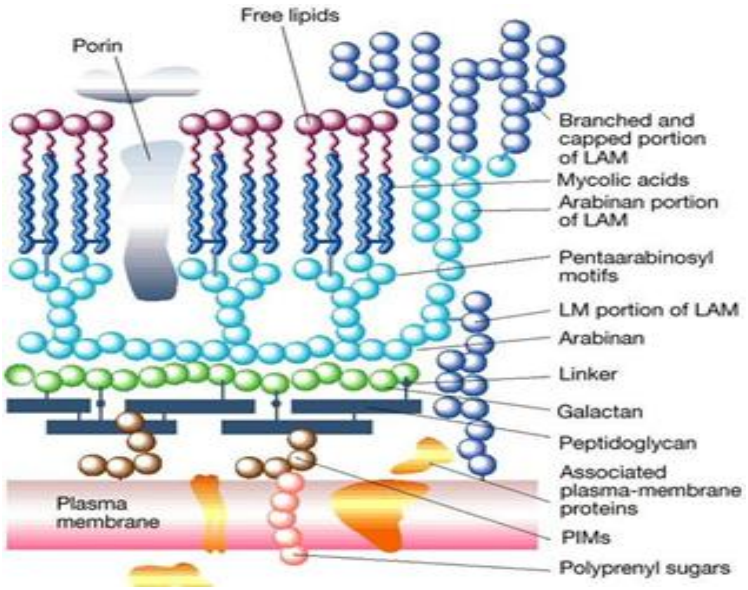


Figure 02: Cell Wall Structure of Mycobacterium tuberculosis[4]

MODE OF TRANSMISSION

When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 μm in diameter. A single sneeze can release up to 40,000 droplets. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very small (the inhalation of fewer than 10 bacteria may cause an infection).

People with prolonged, frequent, or close contact with people with TB are at particularly high risk of becoming infected, with an estimated 22% infection rate. A person with active but untreated tuberculosis may infect 10–15 (or more) other people per year. Transmission occurs from only people with active TB – those with latent infection are not thought to be contagious. The probability of transmission from one person to another depends upon several factors, including the number of infectious droplets expelled by the carrier, the effectiveness of ventilation, the duration of exposure, the virulence of the *M. tuberculosis* strain, the level of immunity in the uninfected person, and others. The cascade of person-to-person spread can be circumvented by effectively segregating those with active ("overt") TB and putting them on anti-TB drug regimens. After about two weeks of effective treatment, subjects with nonresistant active infections generally do not remain contagious to others. If someone does become infected, it typically takes three to four weeks before the newly infected person becomes infectious enough to transmit the disease to others. **[5]**

SIGNS AND SYMPTOMS OF ACTIVE TUBERCULOSIS

1. Coughing that lasts three or more weeks.
2. Coughing up blood.
3. Chest pain or pain with breathing or coughing
4. Unintentional weight loss
5. Fatigue
6. Fever

7. Night sweats and Chills

8. Loss of apetite[6]

EPIDEMIOLOGY

One-third of the world's population is thought to have been infected with *M. tuberculosis*, with new infections occurring in about 1% of the population each year. In 2007, an estimated 13.7 million chronic cases were active globally, while in 2013, an estimated 9 million new cases and 1.5 absolute number of tuberculosis cases has been decreasing since 2006, and new cases have decreased since 2002. The rate of tuberculosis in different areas varies across the globe; about 80% of the population in many Asian and African countries tests positive in tuberculin tests, while only 5–10% of the United States population tests positive. More people in the developing world contract tuberculosis because of a poor immune system, largely due to high rates of HIV infection and the corresponding development of AIDS.

TB disease most commonly affects the lungs; this is referred to as pulmonary TB disease. In 2009, 71% of TB cases in the United States were exclusively pulmonary. Patients with pulmonary TB disease usually have a cough and an abnormal chest radiograph, and may be infectious. Although the majority of TB cases are pulmonary, TB can occur in almost any anatomical site or as disseminated disease.

Persons with LTBI have *M. tuberculosis* in their bodies, but do not have TB disease and cannot spread the infection to other people. In some people, the tubercle bacilli overcome the immune system and multiply, resulting in progression from LTBI to TB disease.

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* has challenged conventional anti-TB therapy and threatens global disease control of TB. The development of new anti-TB drugs is urgently required. β-lactams are effective antibiotics widely used to treat bacterial infections; however, so far no effective anti-TB antibiotics have emerged from this class of drugs. [7] [8]

TYPES OF TUBERCULOSIS:

Tuberculosis is a contagious disease that affects almost all the important organs of the body. Clinically, tuberculosis is broadly categorized into three major categories

Primary Tuberculosis

When tuberculosis affects a person who had never been exposed to the bacterium earlier, the condition is called primary tuberculosis. In this form of tuberculosis, the source of bacterium is external. In primary tuberculosis the lymph nodes get affected leading to their swelling. Lesions are also formed which are removed during treatment. The removal of the lesion does not indicate bacterial removal as the bacteria may have gone into a dormant phase and if left untreated, it can cause TB when favourable condition comes.

Secondary Tuberculosis

It is also known as post-primary tuberculosis. This type of tuberculosis occurs in a person who previously had TB. In primary TB, the bacterium goes into an inactive phase while in secondary tuberculosis; the bacterium regains its active mode and causes the symptoms. Secondary tuberculosis is mostly localized to lungs as oxygen pressure is highest there. Secondary tuberculosis is more infectious than primary tuberculosis. Secondary TB increases the chance of the infection's spread to other organs such as kidneys, heart and brain.

Disseminated Tuberculosis

Disseminated tuberculosis means that the tuberculosis has infected the entire body system. It is a very rare type of disease. Disseminated TB primarily affects the bones of spines, hips, joints and knees, the genital tract of women, the urinary tract and even the central nervous system. It infects the cerebrospinal fluids, the gastrointestinal tract, the adrenal gland, skin of the neck and even the heart.

Miliary Tuberculosis

It is the most severe type of tuberculosis infection. Whole of the blood stream gets infected with the bacterium. Numerous tiny lesions appear throughout the body. If the infection reaches bone marrow, it can cause anaemia. The infection in the blood causes uncontrolled multiplication of white blood cells, thereby leading to leukaemia-like conditions.

Fig 03-Scanning Electron Microscopic Image of *M.tuberculosis*

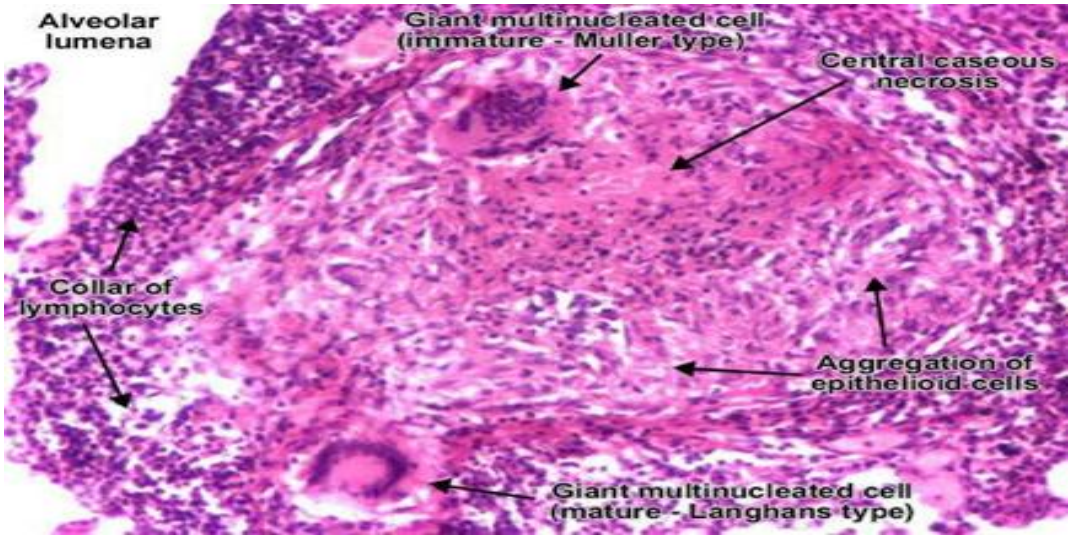


Fig 04- Acid-Fast staining showing caseating granulomas containing Langhans giant cells, which have a "horseshoe" pattern of nuclei

SCIENTIFIC CLASSIFICATION[10]

KINGDOM	Bacteria
PHYLUM	Actinobacteria
CLASS	Actinobacteria
ORDER	Actinomycetales
SUB ORDER	Corynebacterineae
FAMILY	Mycobacteriaceae
GENUS	Mycobacterium
SPECIES	Tuberculosis

GENOME

The genome of the H37Rv strain was published in 1998. Its size is 4 million base pairs, with 3959 genes; 40% of these genes have had their function characterized, with possible function postulated for another 44%. Within the genome are also six pseudogenes.

The genome contains 250 genes involved in fatty acid metabolism, with 39 of these involved in the polyketide metabolism generating the waxy coat. Such large numbers of conserved genes show the evolutionary importance of the waxy coat to pathogen survival.

About 10% of the coding capacity is taken up by the PE/PPE gene families that encode acidic, glycine-rich proteins. These proteins have a conserved N-terminal motif, deletion of which impairs growth in macrophages and granulomas. Nine noncoding sRNAs have been characterized in *M.tuberculosis*, with a further 56 predicted in a bioinformatics screen. [9]

AN INTRODUCTION TO DIAMINOPIMELATE DECARBOXYLASE (LysA)

Introduction

Pyridoxal-dependent decarboxylases that act on ornithine-, lysine-, arginine- and related substrates can be classified into different families on the basis of sequence similarity. One of these families includes ornithine decarboxylase (ODC), which catalyses the transformation of ornithine into putrescine; prokaryotic diaminopimelate decarboxylase, which catalyses the conversion of diaminopimelate into lysine; Pseudomonas syringae pv. tabaci protein, tabA, which is probably involved in tabtoxin biosynthesis and is similar to diaminopimelate decarboxylase; and bacterial and plant biosynthetic arginine decarboxylase, which catalyses the transformation of arginine into agmatine, the first step in putrescine synthesis from arginine.

Although these proteins, which are known collectively as group IV decarboxylases probably share a common evolutionary origin, their levels of sequence similarity are low, being confined to a few short conserved regions. These conserved motifs suggest a common structural arrangement for positioning of substrate and the cofactor pyridoxal 5'-phosphate among bacterial diaminopimelate decarboxylases, eukaryotic ornithine decarboxylases and arginine decarboxylases

This study represents the diaminopimelate decarboxylase LysA, which converts meso-diaminopimelate into lysine and is the last step of the DAP lysine biosynthetic pathway.

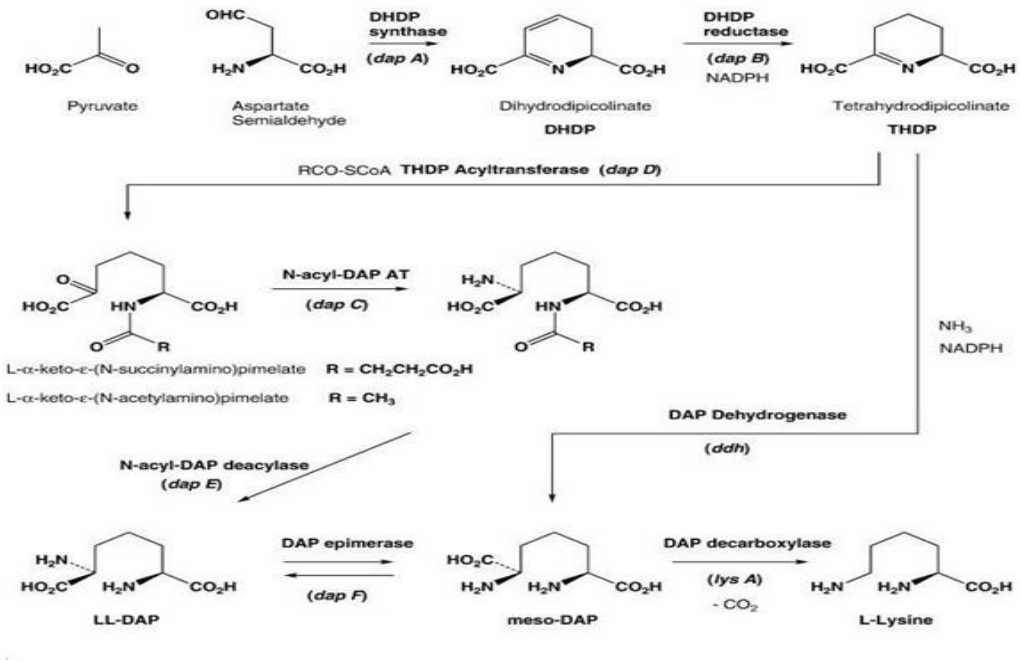


Fig 05- Lysine Biosynthetic Pathway

The *Mycobacterium tuberculosis lysA* gene encodes the enzyme *meso*-diaminopimelate decarboxylase (DAPDC), a pyridoxal-5_-phosphate (PLP)-dependent enzyme. The enzyme catalyzes the final step in the lysine biosynthetic pathway converting *meso*-diaminopimelic acid (DAP) to L-lysine.

meso-2,6-diaminoheptanedioate $\rightleftharpoons$ L-lysine + CO_2

The *lysA* gene of *M. tuberculosis* H37Rv has been established as essential for bacterial survival in immunocompromised mice, demonstrating that *de novo* biosynthesis of lysine is essential for *in vivo* viability. Drugs targeted against DAPDC could be efficient anti-tuberculosis drugs, and the three-dimensional structure of DAPDC from *M. tuberculosis* complexed with reaction product lysine and the ternary complex with PLP and lysine in the active site has been determined. **[11] [12]**

CRYSTAL STRUCTURE OF DIAMINOPIMELATE DECARBOXYLASE

The crystal structure of M. tuberculosis DAPDC confirms its classification as a fold type III B6 dependent enzyme. DAPDC has a fold similar to eukaryotic ODCs (14–16), and DAPDC also forms a stable head-to-tail homodimer of practically identical subunits. Each of the DAPDC subunits (related by proper 2-fold rotation) consists of two ODC-like domains. Domain I is composed of residues 48–308 forming a barrel comprised of α/β barrels comprised of β strands (β4–β13) and helices (α2–α10). The first 47 residues are located in domain II and contain strands , and helix α1, leading into helix α2 of the barrel. The C-terminal domain II contains residues 2–47 (β1, β2, β3, and α1) and 309– 446 (α11–α13, strands β14–β21) and forms a mixed -sheet flanked by β helices. The two structural domains are connected by helix α2 and β13. All of the loops connecting the β strands and α helices were clearly visible in the electron density. Two identical binding sites are formed by residues of both polypeptide chains of the dimer.

The active site is at the interface between the α/β barrel domain of one subunit and the sheet domain of both subunits. Residues from the α/β barrel are mainly involved in binding PLP, whereas residues from the sheet domain primarily contribute to substrate binding. Large conformational changes between the binary DAPDC-lysine and ternary DAPDC-PLP-lysine complex are absent. The only significant differences between the DAPDC complex structures appear near the substrate and cofactor binding sites. **[13]**

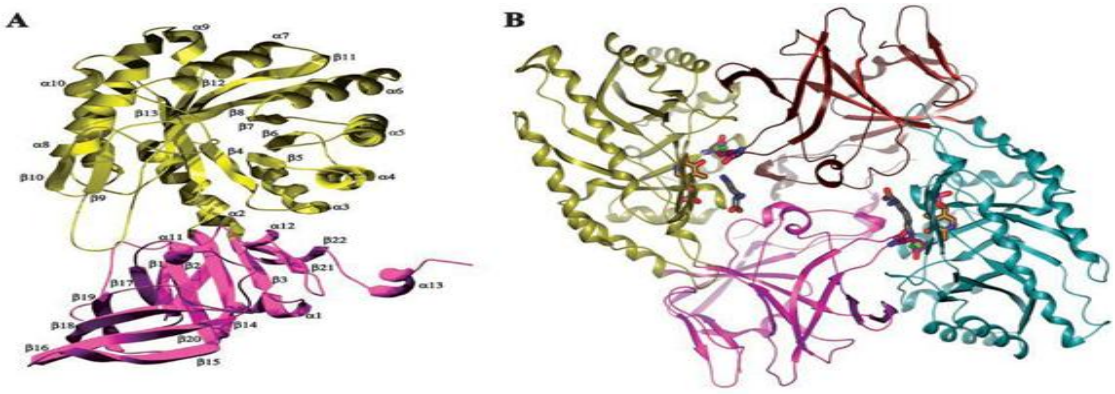


Fig 06-Overview of the *M. tuberculosis* DAPDC structure.

The PLP-binding Site—The active site of *M. tuberculosis* DAPDC is located in a shallow, highly hydrophilic cavity between the dimer interfaces with the deep PLP binding pocket located near the C-terminal ends of the β strands of the α/β barrel, similar to other ODCs.

The oxygen atoms of the PLP phosphate group hydrogen bond with the peptide backbone nitrogen atoms of Gly-258 in the glycine rich motif and those of Gly-302 and Arg-303. OP1 also forms a hydrogen bond with the hydroxyl group of Tyr-405. In the DAPDC-lysine binary complex, a sulfate ion occupies the same position as the phosphate group of PLP in the ternary DAPDC-PLP-lysine structure.

In addition to the covalent link to Lys-72, the pyridyl moiety of PLP is positioned by a hydrogen bond to the side chain carboxylate of Glu-300, which participates in an extended hydrogen bond network with Asp-91 and the conserved residues Asp-254 and His-211.

Lysine Binding to M. tuberculosis DAPDC—In the DAPDC PLP-lysine complex, the density for reaction product lysine could be located in each binding site. In binding site B, the density is very clear and allowed unambiguous positioning and refinement of the lysine molecule. In site A, the lysine is again oriented similarly to the first site, but its exact position along the channel opening in the binding site is not as clear as for site B. Both lysines are positioned with the side chain toward the *si* face of the PLP pyridyl ring, consistent with decarboxylation occurring on this side of the ring.

The carboxyl group of lysine is further fixed by conserved residue Arg-303, which participates in PLP binding via backbone N contacts as well. The ε-amino group and CE of lysine are positioned reasonably close to the catalytic Schiff base formed by the Lys-PLP internal aldimine. A model of the substrate DAP based on the bound lysine would thus have its (D)-aminoacyl group in a position to interact with the internal aldimine from the *si* side of the pyridoxyl ring as well as with conserved His-213, Arg-161, and possibly Ser-377.

ENZYMOLOGY OF DAPDC

GENE NAME	lysA
RV NUMBER	Rv1293
TYPE	CDS
FUNCTION	Involved in biosynthesis of lysine (last step) [catalytic activity: MESO-2,6- diaminoheptanedioate = L-lysine + CO(2)].
PRODUCT	Diaminopimelate decarboxylase LysA (DAP decarboxylase)
FAMILY	Belongs to family 2 of ornithine, DAP, and arginine decarboxylases.
MOLECULAR MASS (Da)	47425.9
ISOELECTRIC POINT	5.0704
GENE LENGTH (bp)	1344
PROTEIN LENGTH	447
LOCATION (kb)	1448.03
FUNCTIONAL CATEGORY	Intermediary metabolism and respiration Identified in the cell wall fraction of M. tuberculosis H37Rv using 2DLC/MS. Identified by mass spectrometry in Triton X-114 extracts of M. tuberculosis
PROTEOMICS	H37Rv. Identified by mass spectrometry in the membrane protein fraction and whole cell lysates of M. tuberculosis H37Rv but not the culture filtrate. Essential gene by Himar1-based transposon mutagenesis in H37Rv strain.
MUTATION	Essential gene for in vitro growth of H37Rv, by sequencing of Himar1-based transposon mutagenesis
PROTEIN DATA BANK	3C5Q
ENZYME CLASSIFICATION	4.1.1.20
GENE ONTOLOGY	Diaminopimelate decarboxylase activity lysine biosynthetic process via diaminopimelate

SELECTION OF DRUG TARGETS

A biological target is a biopolymer such as a protein or nucleic acid whose activity can be modified by an external stimulus. The implication is that a molecule is "hit" by a signal and its behavior is thereby changed. Biological targets are most commonly proteins such as enzymes, ion channels, and receptors.

The "target" is a naturally existing cellular or molecular structure involved in the pathology of interest that the drug-in-development is meant to act on.

The most common drug targets of currently marketed drugs include

- Proteins
  - G protein-coupled receptors (target of 50% of drugs)
  - Enzymes (especially protein kinases, proteases, esterases, and phosphatases)
- Ion channels
  - Ligand-gated ion channels
  - Voltage-gated ion channels
  - Nuclear hormone receptors
  - Structural proteins such as tubulin
  - Membrane transport proteins
  - Nucleic acids

"Established targets" are those for which there is a good scientific understanding, supported by a lengthy publication history, of both how the target functions in normal physiology and how it is involved in human pathology.

"New targets" are all those targets that are not "established targets" but which have been or are the subject of drug discovery campaigns. These typically include newly discovered proteins, or proteins whose function has now become clear as a result of basic scientific research. **[14] [15]**

SCREENING AND DESIGN OF CHEMICAL ENTITIES:

The process of finding a new drug against a chosen target for a particular disease usually involves high-throughput screening (HTS), wherein large libraries of chemicals are tested for their ability to modify the target.

High-throughput screening (HTS) is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry. Through this process one can rapidly identify active compounds, antibodies or genes which modulate a particular biomolecular pathway. The results of these experiments provide starting points for drug design and for understanding the interaction or role of a particular biochemical process in biology.

Another important function of HTS is to show how selective the compounds are for the chosen target. The idea is to find a molecule which will interfere with only the chosen target, but not other, related targets. To this end, other screening runs will be made to see whether the "hits" against the chosen target will interfere with other related targets - this is the process of cross-screening. Cross-screening is important, because the more unrelated target a compound hits, the more likely that off- target toxicity will occur with that compound once it reaches the clinic. **[16] [17]**

DRUG DISCOVERY- FROM HIT TO LEAD

Early drug discovery involves several phases from target identification to preclinical development. The identification of small molecule modulators of protein function and the process of transforming these into high-content lead series are key activities in modern drug discovery. The Hit-to-Lead phase is usually the follow-up of high-throughput screening (HTS). It includes the following steps:

HIT CONFIRMATION PHASE:

- ***Re-testing:*** compounds that were found active against the selected target are re-tested using the same assay conditions used during the HTS.

- ***Dose response curve generation:*** several compound concentrations are tested using the same assay. An IC50 or EC50 value is then generated. Methods are being developed that may allow the reuse of the compound that generated the hit in the initial HTS step.

- ***Orthogonal testing:*** Confirmed hits are assayed using a different assay which is usually closer to the target physiological condition or using a different technology.

- ***Secondary screening:*** Confirmed hits are tested in a functional assay or in a cellular environment. Membrane permeability is usually a critical parameter.

- ***Chemical amenability:*** Medicinal chemists evaluate compounds according to their synthesis feasibility and other parameters such as up-scaling or costs

- ***Intellectual property evaluation:*** Hit compound structures are quickly checked in specialized databases to define patentability

- ***Biophysical testing:*** Nuclear magnetic resonance (NMR), Isothermal Titration Calorimetry, dynamic light scattering, surface Plasmon resonance, dual polarisation interferometry, microscale thermophoresis (MST) are commonly used to assess whether the compound binds effectively to the target, the stoichiometry of binding, any associated conformational change and to identify promiscuous inhibitors.

- ***Hit ranking and clustering:*** Confirmed hit compounds are then ranked according to the various hit confirmation experiments. **[18]**

HIT EXPANSION

Following hit confirmation, several compound clusters are chosen according to their characteristics in the previously defined tests. An ideal compound cluster will:

- Have compound members that exhibit a high affinity towards the target (less than 1 µM)
- Moderate molecular weight and lipophilicity (usually measured as cLogP). Affinity, molecular weight and lipophilicity can be linked in single parameter such as ligand efficiency and lipophilic efficiency to assess druglikeness
- Showed chemical tractability
- Be free of Intellectual property
- Showed not interfere with the P450 enzymes nor with the P-glycoproteins
- Showed not bind to human serum albumin
- Be soluble in water (above 100 µM)
- Be stable
- Have a good druglikeness
- Exhibit cell membrane permeability
- Showed significant biological activity in a cellular assay
- Showed Not exhibit cytotoxicity
- Showed Not be metabolized rapidly
- Showed selectivity versus other related targets[18]

LEAD OPTIMIZATION PHASE

The objective of this drug discovery phase is to synthesize lead compounds, new analogs with improved potency, reduced off-target activities, and physiochemical/metabolic properties suggestive of reasonable in vivo pharmacokinetics. This optimization is accomplished through chemical modification of the hit structure, with modifications chosen by employing knowledge of the structure-activity relationship (SAR) as well as structure-based design if structural information about the target is available. [18]

DRUG LIKENESS

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A druglike molecule has properties such as:

Solubility in both water and fat, as an orally administered drug needs to pass through the intestinal lining after it is consumed, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of a cell. A model compound for the lipophilic cellular membrane is octanol (a lipophilic hydrocarbon), so the logarithm of the **octanol/water partition coefficient**, known as **LogP**, is used to predict the solubility of a potential oral drug. This coefficient can be experimentally measured or predicted computationally, in which case it is sometimes called **"cLogP**".

Potency at the target of interest. High potency (high value of pIC_{50}) is a desirable attribute in drug candidates, as it reduces the risk of non-specific, off-target pharmacology at a given concentration. When associated with low clearance, high potency also allows for low total dose, which lowers the risk of idiosyncratic drug reactions.

Several scoring methods can be used to express druglikeness as a function of potency and physicochemical properties, for example ligand efficiency and lipophilic efficiency.

Since the drug is transported in aqueous media like blood and intracellular fluid, it has to be sufficiently water-soluble. Solubility in water can be estimated from the number of hydrogen bond donors vs. alkyl side chains in the molecule. Low water solubility translates to slow absorption and action. Too many hydrogen bond donors, on the other hand, lead to low fat solubility, so that the drug cannot penetrate the cell wall to reach the inside of the cell.

Molecular weight: The smaller the better, because diffusion is directly affected. Eighty percent of traded drugs have molecular weights under 450 daltons; they belong to the group of small molecules.

Substructures that have known chemical or pharmacological properties. For example, alkyl nitro compounds tend to be irritants. **[19] [20] [21]**

LIPINSKI'S RULE OF FIVE

Lipinski's rule of five also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A Lipinski in 1997.

Lipinski et al, has proposed **rule of five** which describes the molecular properties that are important for a drugs pharmacokinetics (ADME). The rule has been summarized below:

- Molecular Weight less than 500 Daltons
- Calculated log P value should be less than 5
- Less than 10 hydrogen bond acceptor groups (e.g.: -O-, -N-, etc.)
- Less than 5 hydrogen bond donar groups (e.g.: OH, NH, etc.)
- Less than 10 rotatable bonds

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active. **[22] [23] [24]**

PHARMACOPHORE MODELING

Pharmacophore approaches have become one of the major tools in drug discovery. Various ligand-based and structure-based methods have been developed for improved pharmacophore modeling and have been successfully and extensively applied in virtual screening, de novo design and lead optimization.

Historically, pharmacophores were established by Lemont Kier, who first mentions the concept in 1967. A **pharmacophore** is a description of molecular features which are necessary for molecular recognition of a ligand by a biological macromolecule. The IUPAC defines a pharmacophore to be *"an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response".*

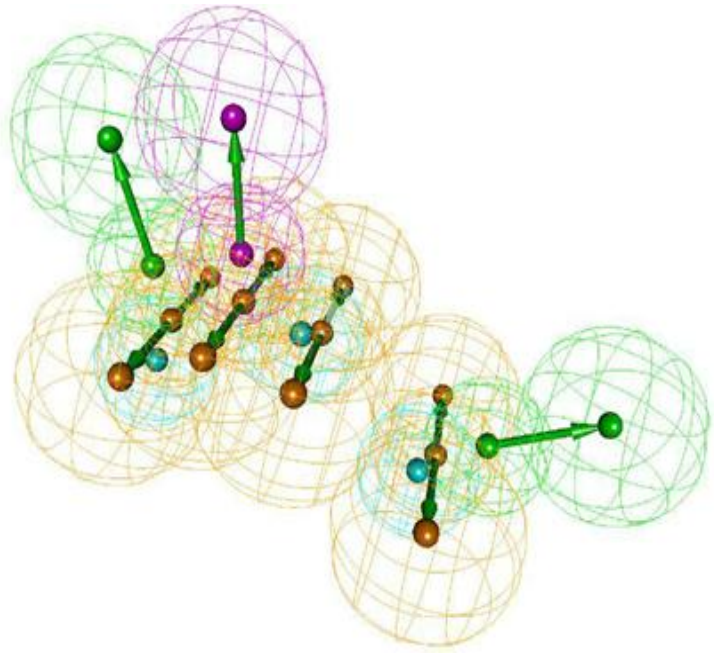


Fig 07 - An example of a pharmacophore model.

Typical pharmacophore features include hydrophobic centroids, aromatic rings, hydrogen bond acceptors or donors, cations, and anions. These pharmacophoric points may be located on the ligand itself or may be projected points presumed to be located in the receptor.

The features need to match different chemical groups with similar properties, in order to identify novel ligands. Ligand-receptor interactions are typically "polar positive", "polar negative" or "hydrophobic". A well-defined pharmacophore model includes both hydrophobic volumes and hydrogen bond vectors. **[24] [25]**

HISTORY OF CHEMOTHERAPY OF TUBERCULOSIS

The present chemotherapy treatment for tuberculosis is one of the most spectacular achievements of medicine. Since 50 years ago, when the only effective treatment methods were surgically collapsing the lung and sanatoriums, we have advanced to a very different treatment of drug regimens which are easy to use, have low toxicity, and are effective in every case. The main goal in treating Tuberculosis was to avoid hindering natural cures. Continual rest, a balanced diet, and abstaining from anything in excess including sex were considered crucial. A well-balanced diet and drinking a large amount of milk from a cow, goat, or woman by itself or mixed with honey, were also important. For centuries, milk was practically considered the cure for tuberculosis.

During the 16th and 17th centuries, sulfur, arsenic, mercury, and every type of plant from the New World such as quinine (tea), cocoa, and tobacco were all thoroughly tested and none were found to be useful.

An interesting therapy called "cure by regal touch" was started in the middle Ages and persisted until the 19th century. This privilege given to some kings (especially French and English) was to cure certain illnesses by placing their hands over the patient while reciting the phrase "the king touches you and God cures you."

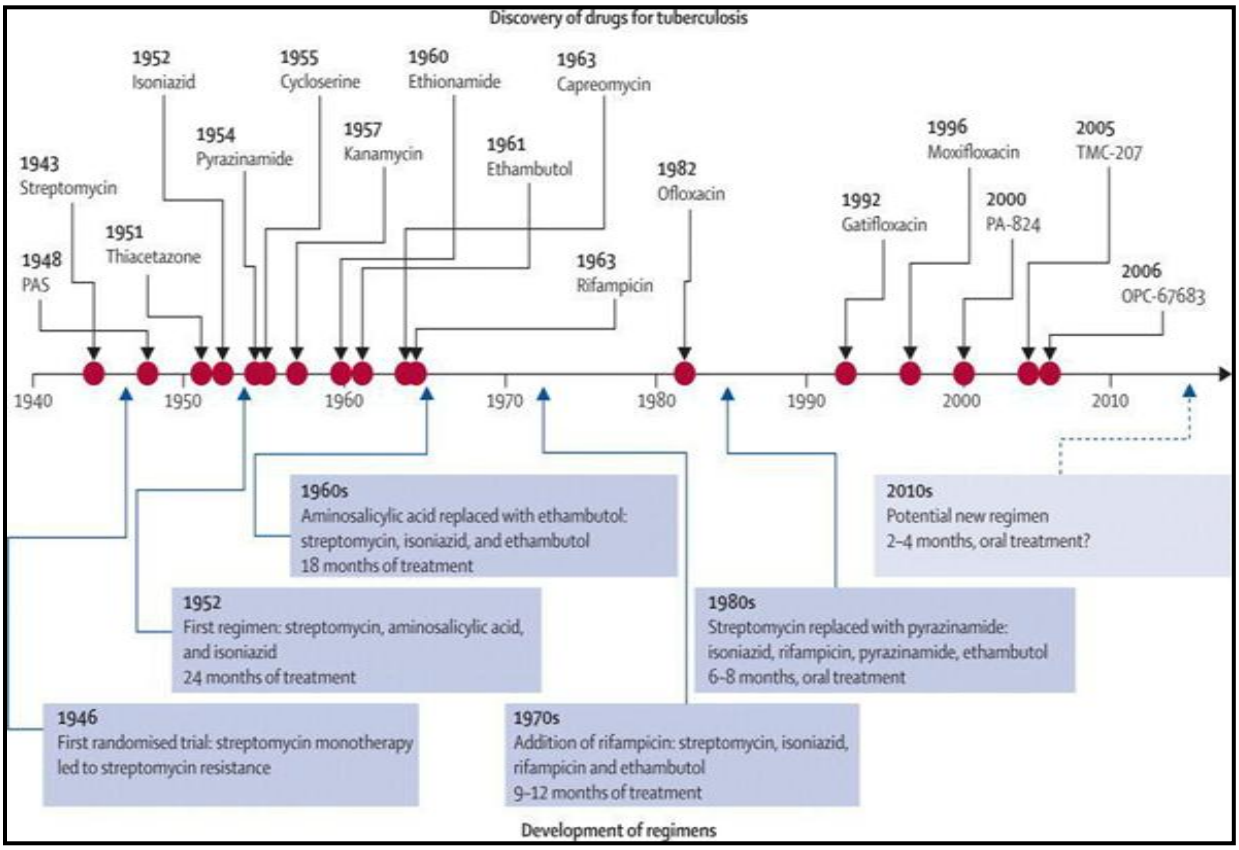
In 1920, a microbiologist, A. Calmette, and his veterinarian student, C. Guerin, spent three years cultivating bacteria and eventually found a bovine species of bacteria with rare virulence that could possibly be developed into active immunity against tuberculosis.

The **BCG vaccine** was a great hope to many but because of its widespread use, actually became an obstacle for the powerful impact of modern chemotherapy

A few years after the discovery of penicillin, Selman A. Waksman demonstrated that small fungi of the genus Streptomyces griseus inhibit the growth of M.tuberculosis cultures because of a substance called Streptomycin. The massive industrial production of streptomycin in 1946 produced the first effective chemotherapy against tuberculosis.

J. Lehman synthesised para-amino-salicylic acid in 1944 and later discovered isonaizid, a hydrazine from isonicotinic acid. Administration of isonaizid with streptomycin solved the problem of resistance and finally achieved the long-lived dream of mankind: to find a chemotherapy treatment to cure all the cases and visceral regions of tuberculosis. Despite its advantages, a serious inconvenience to the effectiveness of the treatment was the need to administer the drugs for 12-18 months to ensure sterilization.

In 1966, the Italian Pietro Sensi isolated Rifamycin S from fungus of the genus Streptomyces mediterranii. It was supposedly a new treatment revolution to be verified upon experimental studies because its actions against all types of bacteria complimented the specific activity of pyrazinamide against intercellular bacteria. These findings were the basis of shortened chemotherapy treatment of 6 months. They were tested in a clinical study in east Africa between 1972 and 1976 and were recommended as initial treatment for tuberculosis. **[26]**



History of Tuberculosis Drug Discovery

THE NEED FOR NOVEL TUBERCULOSIS DRUGS [27] [28] [29]

- To improve current treatment by shortening the total duration of treatment.
- To provide more effective treatment of latent tuberculosis infection.
- New drugs to improve current drugs that facilitate compliance by providing less intensive supervision are also of great interest.
- Discovery of a compound that would reduce both the total length of treatment and the frequency of drug administration.
- Emergence of MDR Tb, XDR Tb and TDR Tb needs to be attended to with never molecules.

2. AIM AND OBJECTIVE OF THE STUDY

AIM

The aim is to design and synthesis never antitubercular agents which will be more potent and possess less side effects and effective against resistant form of Tb.

OBJECTIVE

The present study relates to the synthesis of various *pyridine-4-carbohydrazide* and *pyridine-3-carbohydrazide* derivatives and subsequent screening for their anti-tubercular activity. Due to several toxic effects of isoniazid, attempts were made to eliminate the toxicophore and substituting with a group contributing to the anti-tubercular action. This work also aims the same motive and the compounds were synthesized according to the developed and valid synthetic route.

The plan of work includes: Design of DiAminoPimelate DeCarboxylase (DAPDC) inhibitors by docking studies using Argus lab 4.0 software.

The present study carried out based on the following design.

Identification of the lead compound

↓

Lead optimization

↓

Docking of the molecule to the target protein

↓

Top docking score compounds selected

↓

IN-SILICO drug likeness and toxicity risk prediction

↓

Synthesis

↓

Justification of purity-TLC, MP,GC

↓

Characterization – Spectroscopy (IR, NMR,GC-MS and LC-MS)

↓

IN-VITRO anti-tubercular activity

↓

Results and Discussion

↓

Summary and Conclusion

DOCKING

Several chemical libraries containing various scaffolds will be sketched and docked against the 3D structure of DAPDC. The compounds for the synthesis were chosen based on the high G-Score and their feasibility in synthetic chemistry.

3. REVIEW OF LITERATURE

The purpose of a literature review is to:

- Establish a theoretical framework for a topic / subject area
- Define key terms, definitions and terminology
- Identify studies, models, case studies etc supporting a topic
- Define / establish an area of study.

The following works throw light upon the various genomic aspects of *M.tuberculosis* and also various targets intended for drug action

RELATED TO GENOMICS ASPECTS

- **Ashok Rattan *et al* (1998)** published his work on Multidrug-Resistant *Mycobacterium tuberculosis*: Molecular Perspectives. [30]
- **Puneet Chopra *et al* (2003)** reported New drug targets for *Mycobacterium tuberculosiss.*[31]
- **James C Sacchettini *et al* (2003)** reported Mycobacterium tuberculosis: a model system for structural genomics.[32]
- **R. Hernandez Pando *et al* (2006)** published their work on The use of mutant mycobacteria as new vaccines to prevent tuberculosis. [33]
- **Khisimuzi Mdluli and Melvin Spigelman (2006)** reported Novel targets for tuberculosis drug discovery.[34]
- **Johan Weigelt *et al* (2008)** published their work correlating Structural genomics and drug discovery: all in the family. [35]

- **Yee Siew Choong (2011)** reported the Effects of Enoyl-Acyl Protein Carrier Reductase Mutations on Physiochemical Interactions with Isoniazid: Molecular Dynamics Simulation. [36]

- .**T. Cole et al (2012)** worked on Isolation and characterization of isoniazid-resistant mutants of *Mycobacterium smegmatis* and *M. aurum.* [37]

- **Dorothy Yeboah-Manu *et al* (2014)** conducted a study on Drug Susceptibility Pattern of Mycobacterium Tuberculosis Isolates From Ghana; Correlation with Clinical Response. [38]

RELATED TO TARGETS

- ***Soumya S. Ray et al (2002)*** worked on Co crystal Structures of Diaminopimelate Decarboxylase: Mechanism, Evolution, and Inhibition of an Antibiotic Resistance Accessory Factor. [39]

- ***Kuppan Gokulan et al (2003)*** reported Crystal Structure of *Mycobacterium tuberculosis* Diaminopimelate Decarboxylase, an Essential Enzyme in Bacterial Lysine Biosynthesis. [40]

- ***Simone Weyand et al (2009)*** reported The three-dimensional structure of diaminopimelate decarboxylase from Mycobacterium tuberculosis reveals a tetrameric enzyme organization. [41]

- ***Viola RE et al (2011)*** reported The catalytic machinery of a key enzyme in amino Acid biosynthesis. [42]

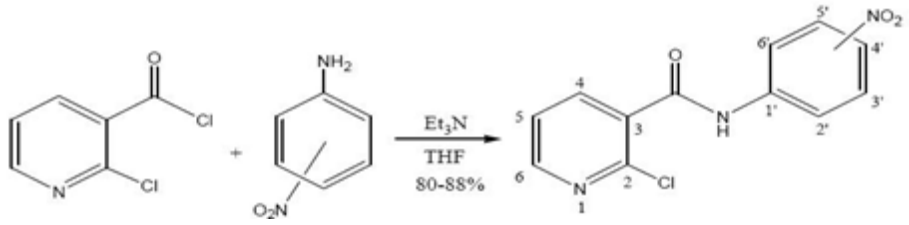
- ***Sakshi Kohli et al (2012)*** Comparative genomic and proteomic analyses of PE/PPE multigene family of *Mycobacterium tuberculosis* $H_{37}Rv$ and $H_{37}Ra$ reveal novel and interesting differences with implications in virulence. [43]

RELATED TO SYNTHESIS

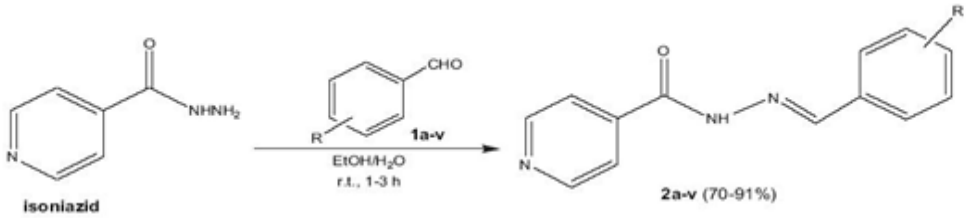
- ***Jørn B. Christensen (2001)*** carried out a A Simple Method for Synthesis of Active Esters of Isonicotinic and Picolinic Acids. [44]

- ***Suriyati Mohamad et al (2004)*** studied the Susceptibility of *Mycobacterium tuberculosis* to isoniazid and its derivative, 1-isonicotinyl-2-nonanoyl hydrazine: investigation at cellular level. [45]

- ***Marcus V. N. de Souza et al (2007)*** Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. [46]



- **Marcus Vinı´cius Nora de Souza (2008)** carried out Synthesis and anti-mycobacterial activity of (E)-N0-(monosubstituted-benzylidene) isonicotino hydrazide derivatives. [47]



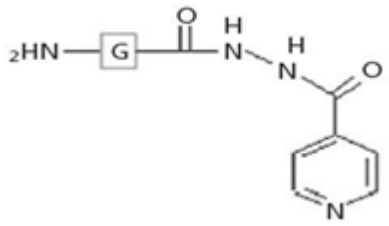
- ***R.P. Tripathi (2009)*** worked on Design and Development of New Generation of Antitubercular Agents. [48]

- ***Miyeon Jang (2009)*** established Synthesis and biological evaluation of bicyclic heterocycles. **[49]**

- ***Mauro V. de Almeida et al (2009)*** Synthesis and antitubercular activity of isoniazid condensed with carbohydrate derivatives. **[50]**

- ***Marcus V.N. de Souza et al (2010)*** carried out Synthesis and Antitubercular Activity of Heteroaromatic Isonicotinoyl and 7-Chloro-4-Quinolinyl Hydrazone Derivatives. **[51]**

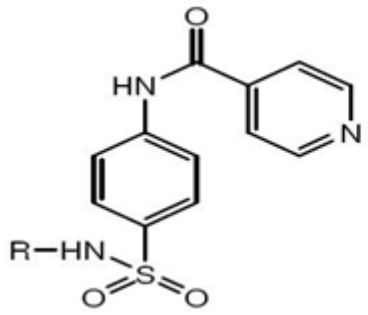
- ***Roberta Cassano et al (2011)*** reported Synthesis, characterization and in-vitro antitubercular activity of isoniazid-gelatin conjugate. [52]

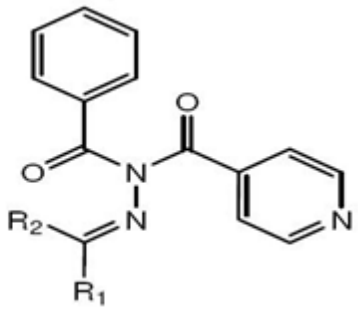


- **Jahnavialuri (2011)** carried out Synthesis of Certain Derivatives of Schiff bases of Isoniazid and Its in-Vitro Assay against Tuberculosis - Multi and Extremely Drug Resistance Strains. **[53]**

- **Vikramjeet Judge *et al* (2011)** worked on Isonicotinic acid hydrazide derivatives: synthesis, antimicrobial activity, and QSAR studies. **[54]**

- **C.N.Nalini *et al* (2011)** worked on Structure Based Drug Design, Synthesis, Characterization And Biological Evaluation Of Novel Isoniazid Derivatives. **[55]**





- ***Anu kajal et al (2013),*** Schiff Bases: Schiff Bases : A Versatile Pharmacophore.[56]

- ***Ruchi Agarwal et al (2013),*** Schiff base complexes derived from thiosemicarbazone, synthesis characterization and their biological activity. [57]

RELATED TO MICROPLATE ALAMAR BLUE ASSAY

- ***Page et al (1993)*** conducted A New Fluorometric Assay for Cytotoxicity Measurements InVitro. [58]

- ***Geier, Steven (1994)*** published his work on Analysis of alamar Blue Overlap: Contribution of Oxidized to Reduced. [59]

- ***Lancaster, M.V. and Fields, R.D. (1996)*** carried out Antibiotic and Cytotoxic Drug Susceptibility Assays using Resazurin and Poising Agents. [60]

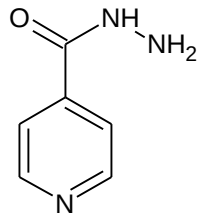
- ***R Hamid et al (2004)*** carried out Comparison of alamar blue and MTT assays for high through-put screening. [61]

- ***C. N. Paramasivan et al (2004)*** carried out Evaluation of microplate Alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. [62]

4. MATERIALS AND METHODOLOGY

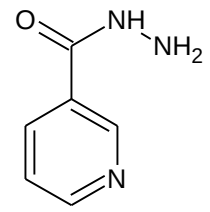
REACTANT PROFILE

ISONIAZID



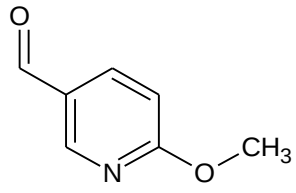
Molecular Formula	:	C6H7N3O
Molecular Weight	:	137.13 g/Mol
Description	:	White Crystalline solid
Melting point	:	169°C-174°C
Solubility	:	Soluble in water, methanol, ethanol

NICOTINIC ACID HYDRAZIDE



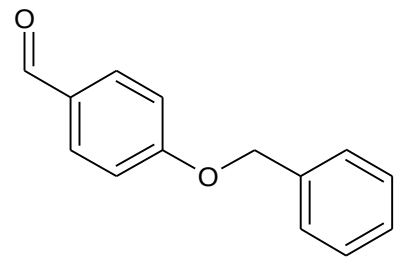
Molecular Formula	:	C6H7N3O
Molecular Weight	:	137.13 g/Mol
Description	:	White Crystalline solid
Melting point	:	160°C-163°C
Solubility	:	Soluble in water, methanol, ethanol

PYRIDINE -2-METHOXY-5-CARBOXALDEHYDE



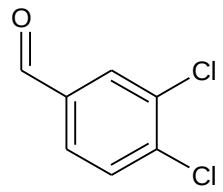
Molecular Formula	:	C7H7NO2
Molecular Weight	:	137.13 g/Mol
Description	:	Off White to light yellow Crystalline powder
Melting point	:	51°C-54°C
Solubility	:	Soluble in methanol,ethanol. Insoluble in water

4-BENZYLOXY BENZALDEHYDE



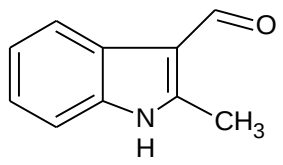
Molecular Formula	:	C14H12O2
Molecular Weight	:	212.24 g/Mol
Description	:	Creamish to Yellow Crystalline Powder
Melting point	:	71°C-74°C
Solubility	:	Soluble in methanol, ethanol. Insoluble in water

3,4 DICHLORO BENZALDEHYDE



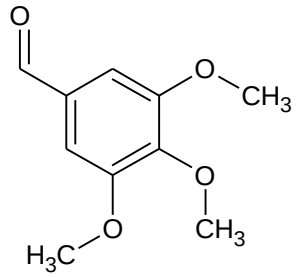
Molecular Formula	:	C7H4Cl2O
Molecular Weight	:	175.01 g/Mol
Description	:	White Crystalline solid
Melting point	:	43°C-45°C
Solubility	:	Soluble in methanol,ethanol. Insoluble in water

2-METHYL INDOLE-3-CARBOXALDEHYDE

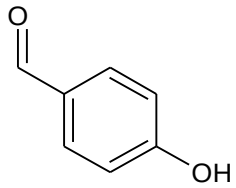


Molecular Formula	:	C10H9NO
Molecular Weight	:	159.18 g/Mol
Description	:	Brownish White powder
Melting point	:	204°C-205°C
Solubility	:	Soluble in water, methanol, ethanol

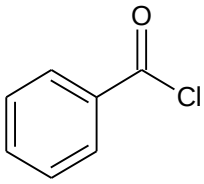
3,4,5 TRIMETHOXY BENZALDEHYDE

	Molecular Formula	:	C10H12O4
	Molecular Weight	:	196.19 g/Mol
	Description	:	Light Yellowish solid
	Melting point	:	73°C-75°C
	Solubility	:	Soluble in methanol,ethanol. Slightly soluble in water.

P-HYDROXY BENZALDEHYDE

	Molecular Formula	:	C7H6O2
	Molecular Weight	:	122.12 g/Mol
	Description	:	Yellowish powder
	Melting point	:	112°C-116°C
	Solubility	:	Soluble in water, methanol, ethanol

BENZOYL CHLORIDE

	Molecular Formula	:	C7H5ClO
	Molecular Weight	:	140.56 g/Mol
	Description	:	Colorless Fuming liquid
	Melting point	:	197.2°C
	Solubility	:	Soluble in organic liquids, reacts with water

Docking program is used to fit the ligand molecules into the target structure in a variety of positions, conformations, and orientations. Docking mode is known as pose. Each pose is scored based on its complementarity to the target in terms of shape and properties such as electrostatics in order to identify the most favorable energetical pose.

The quality of any docking result depends on the starting structure of both the protein and the potential ligand. The protein and ligand structures need to be prepared to achieve the best docking results. **[63]**

MOLECULAR DOCKING BY ARGUS LAB 4.0

Argus lab 4.0 is distributed freely for windows platforms by planaria software. It is an introductory molecular modeling package with academics. Argus lab approximates an exhaustive search method which is similar to DOCK and GLIDE. Flexible ligand docking is possible with Argus lab, where the ligand is described as torsion tree or free and grids are constructed that overlay the binding site. The accuracy of the Argus lab docking algorithm takes into account, the key features such as the nature of the binding site and the number of rotatable bonds to the ligand. **[64]**

MOLEGRO® MOLECULAR VIEWER

Molegro® molecular viewer is an application which helps in analyzing the energies and interaction of the binding site.

Q-site finder

Q-site finder is an energy-based method for protein-ligand binding site prediction. During prediction we use the crystal structures of macromolecules (receptor) with small substrates (pdb ID). Identifying the location of binding sites on a protein is of fundamental importance for a range of applications including molecular docking. It uses the interaction energy between the protein and a simple vanderwaals probe to locate energetically favourable binding sites. **[65]**

STEPS INVOLVED IN DOCKING PROCEDURES

A.PREPARATION OF PROTEIN

B.IDENTIFICATION/ SELECTION OF ACTIVE SITE

C.PREPARATION OF LIGANDS

D.DOCKING PARAMETER

E.VISUALIZATION/INTERPRETATION OF DOCKING

A.PREPARATION OF PROTEIN

Enter protein pdb ID (3C5Q) in the protein data bank and downloaded the protein pdb ID as a text file and saved to the desktop. Then opened Argus Lab file imported pdb file from the desktop. 3D structure of the protein appeared in the workspace of Argus Lab.Later opened the pdb ID, residues and miscellaneous. From miscellaneous deleted the inhibitors and hetero residues, but not deleted cofactor. Afterwords all the water molecules were deleted and added hydrogen atoms. Later opted energy by Universal Force Field (UFF) method and started the calculation. The prepared protein saved as *.agl file format in the desktop.

B.IDENTIFICATION/ SELECTION OF ACTIVE SITE

Open Q-site finder opened through online, imported the pdb format of the protein and selected all the active amino acids site from the list of amino acids. The selected amino acids residues were grouped as in the name of ‘Binding Site’

C.PREPARATION OF LIGAND

The ligand drawn in Chem sketch and saved it as MDL mol file format and it imported into the workspace of the Argus Lab. The ligand were prepared by cleaning the geometry and hybridization, then it grouped as in the name of ‘Ligand’ Import the ligand into workspace of Argus lab.

D.DOCKING PARAMETER

From the calculation of the tool bar 'Argus Dock' selected as the Docking engine. Dock were selected as calculation type and 'Flexible' for the ligand. Then started the docking and the docked. Docked protein ligand compex saved as Brookhaven pdb files (*.pdb).

E.VISUALIZATION/INTERPRETATION OF DOCKING

Molegro molecular viewer will help in analyzing The energies and interaction of the Protein-Ligand binding viewed and analysed by Molegro® Molecular Viewer.

SCORING FUNCTION

These are mathematical methods used to predict the strength of the non-covalent interaction called as binding affinity, between the two molecules after they have been docked. Scoring functions have also been developed to predict the strength of other types of intermolecular interactions, for sample between two proteins or between protein and DNA or protein and drug. These configurations are evaluated using scoring functions to distinguish the experimental binding modes from all other modes explored through the searching algorithm. **[66]**

PREDICTION OF ADME

ADME acronym is used to indicate phenomenon associated with Absorption, Distribution, Metabolism and Elimination. Therefore, a full consideration of molecular structure and their impact on ADME profile will enable the chemists to eliminate negative ADME attributes (e.g. chemically active moiety) and incorporate desirable ADME attributes (e.g. optimal log P, good membrane permeability, etc.). Hence the prediction is crucial to the drug development process. In recent years, many insilico tools are available to determine those properties. **[67]**

INSILICO SCREENING OF DRUG LIKENESS

The designed and docked molecules are screened insilico using **Molinspiration cheminformatics software** to evaluate drug likeness. Molinspiration supports internet chemistry community by offering free on-line services for calculation of important molecular properties such as logP, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioactivity score for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors.

INSILICO TOXICITY PREDICTION

Toxicity is one of the major criteria to be considered for a molecule to shine as a successful clinical candidate in pharmaceutical research. About 20-40% of drug failure comes under this category. Commercial insilico tools estimates toxicity and provides information by the use of QSAR, scientific literatures and to some extent in abstracting issues from humans. **[68]**

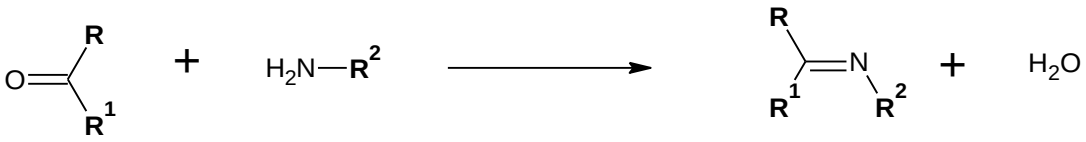
Toxicity screening is done insilico using **OSIRIS Property Explorer.** The OSIRIS Property Explorer lets us to draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behavior**.** **[69] [70]**

SYNTHETIC METHODOLOGY

DRUG PROFILE

SCHIFF BASE

Schiff bases are the compounds carrying imine or azomethine (–C=N–) functional group. These are the condensation products of primary amines with carbonyl compounds and were first reported by Hugo Schiff. Schiff bases form an important class of the most widely used organic compounds and has a wide variety of applications in many fields including analytical, biological, and inorganic chemistry. Schiff bases have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like anti-inflammatory analgesic, antimicrobial, anticonvulsant, anti tubercular, anticancer, antioxidant, anthelmintic, and so forth. The nitrogen atom of azomethine may be involved in the formation of a hydrogen bond with the active centers of cell constituents and Interferes in normal cell processes. Apart from biological activities, Schiff bases are also used as catalysts, intermediates in organic synthesis, dyes, pigments, polymer stabilizers, and Corrosion inhibitors. Schiff base derivatives in various processes promoted the researchers for designing of novel heterocyclic/aryl Schiff bases for development of new environmental friendly technology.[71]

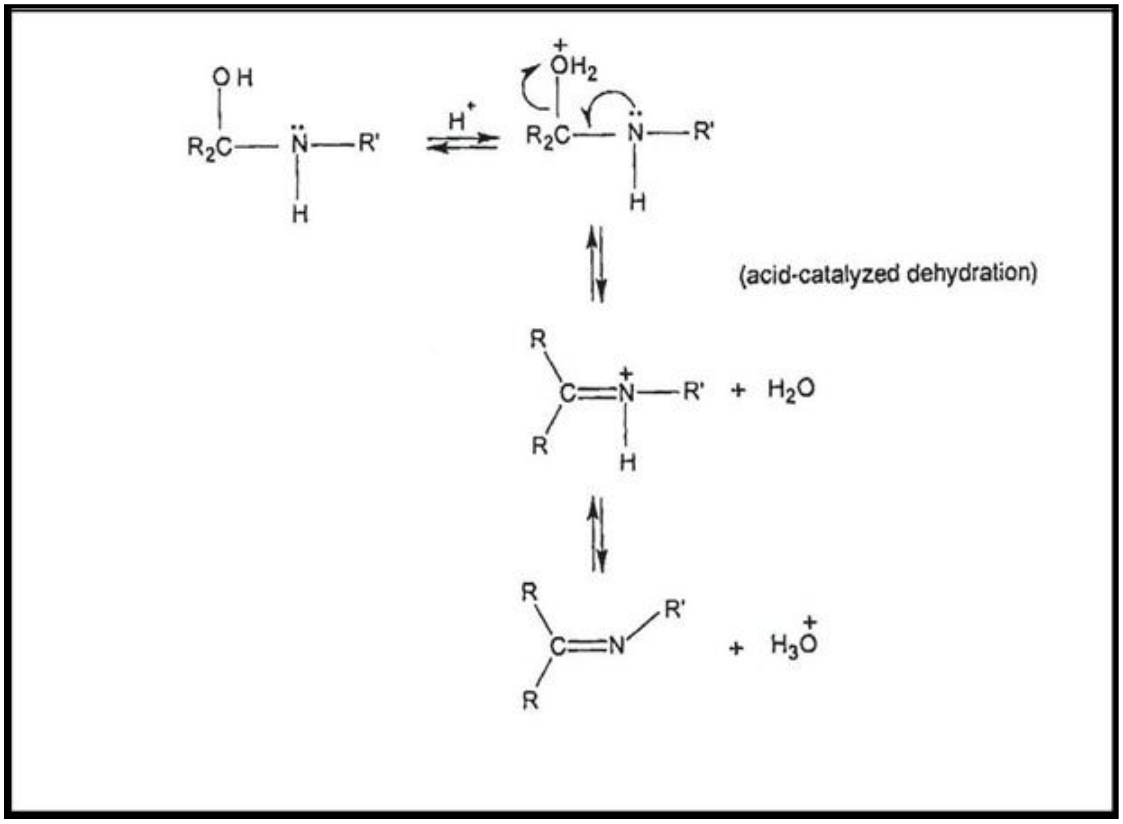


MECHANISM

Schiff base formation involves nucleophilic addition to the carbonyl group. In this case, the nucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed

dehydration. Typically the dehydration of the carbinolamine is the rate-determining step of Schiff base formation and that is why the reaction is catalyzed by acids.

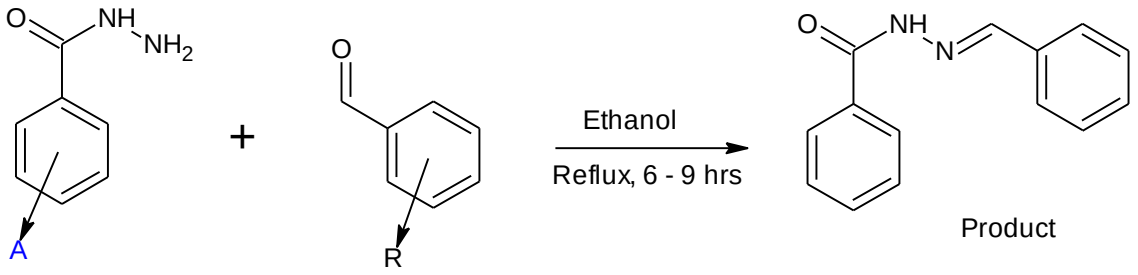
The Schiff base formation is really a sequence of two types of reactions, i.e. ***addition*** followed by ***elimination*** [72]



PROCEDURE: Synthesis of Schiff bases [73]

SCHEME I

The Compound of Schiff base was synthesized by refluxing ethanolic solution of Acid hydrazide (1mMol) with Aromatic/ Hetero aromatic aldehyde **(R)** (1mMol) in ethanol for 6-9 hrs. The refluxed mixture was poured in crushed ice and stirred well, The precipitate was filtered, dried and recrystallised from hot ethanol to yield a crystal form of Schiff base.



Acid hydrazides (A) used:

- Isonicotinic acid hydrazide (INH),
- Nicotinic acid hydrazide (NH)

Aldehydes(R)used:

- Pyridine-2-methoxy-5-carboxaldehyde
- 4-Benzyloxy Benzaldehyde
- 3,4 Dichloro benzaldehyde
- 2-Methyl indole-3- carboxaldehyde
- 3,4,5-Trimethoxy benzaldehyde
- P-Hydroxy benzaldehyde

SCHEME-II

SYNTHESIS OF N-benzoyl-N'-[(E)-(6-methoxypyridin-3- yl)methylidene] pyridine-3-carbohydrazide *(RK 2a)*

STEP: 1

The Compound of Schiff base was synthesized by refluxing ethanolic solution of nicotinic acid hydrazide (1mMol) with Aromatic/ Hetero aromatic aldehyde **(R)** (1mMol) in ethanol for 6-9 hrs. The refluxed mixture was poured in crushed ice and stirred well, The precipitate was filtered, dried and recrystallised from hot ethanol to yield a crystal form of Schiff base.

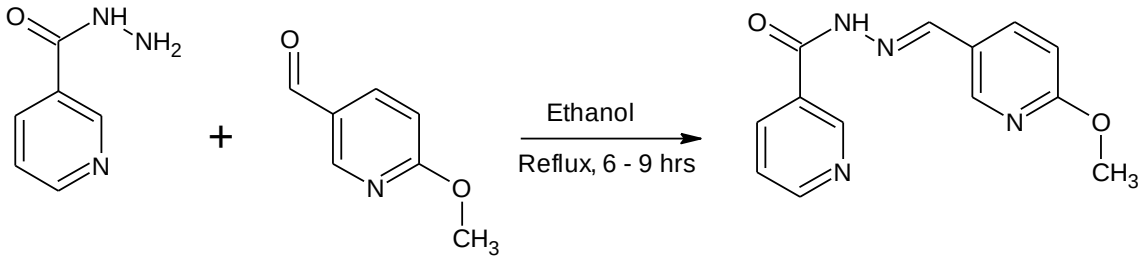
STEP: 2

About 1 mMol of schiff base was suspended in 20 mL of dichloromethane and was stirred mechanically. To this, was added 1 mMol of benzoyl chloride dropwise for over 15 mins and the stirring was continued for 4 - 6 hrs. The reaction mixture was subjected to TLC to ensure the progress of the reaction. After 4 - 6 hrs, the stirring was stopped and the solvent was allowed to evaporate. The solid mass was recrystallised from ethanol:dioxane (1:1) to yield pale yellow crystals.

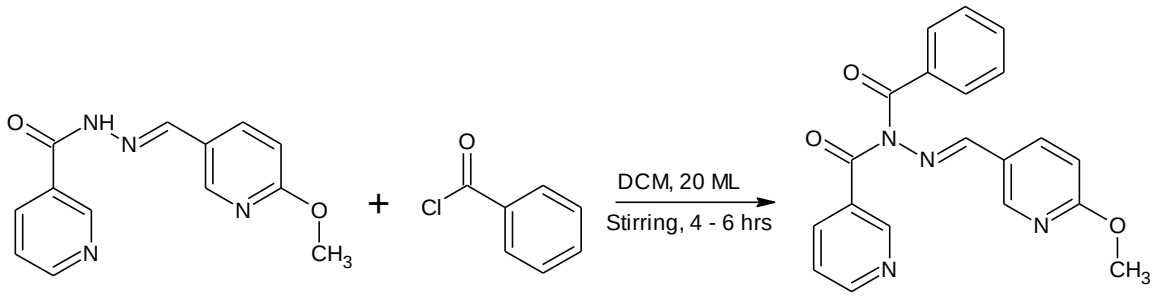
SYNTHETIC ROUTE FOR SCHEME II

SYNTHESIS OF N-benzoyl-N'-[(E)-(6-methoxypyridin-3- yl)methylidene] pyridine-3-carbohydrazide *(RK 2a)*

STEP: 1



STEP: 2



RK 2a

JUSTIFICATION OF PURITY

MELTING POINT

The melting point of the synthesized compound was determined by one end open capillary method. The melting points were sharp and were presented corrected.

THIN LAYER CHROMATOGRAPHY

Precoated aluminium TLC plates were used. Solutions of the reactants and products were prepared by dissolving them in methanol.

Stationary Phase	:	Precoated Silica Gel Plates
Mobile Phase	:	Chloroform:Acetone:Methanol (8:1:1)
Visualization	:	Iodine Vapors and UV chamber

A single spot not corresponding to the parent compound was noticed and hence the purity of the synthesized compounds was justified.

CHARACTERIZATION

IR SPECTROSCOPY

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists. The main goal IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

The synthesized compounds were made into pellets with potassium bromide by pressed pellet technique using pellet press (model No: M15). The pellet was mounted on the pellet disc and percentage transmittance was recorded in ABB IR Spectrophotometer (Model No: MB 3000).

NMR SPECTROSCOPY

Proton NMR Spectroscopy helps us to study the number of equivalent protons and their environment thereby we can ascertain the structure of the molecule. The NMR spectra were recorded on 300 MHz BRUKER Advance III NMR Spectrometer. DMSO was used as solvent.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Gas chromatography-mass spectrometry (GC-MS) is a **hyphenated** technique; consisting of two analytical procedures in sequence, namely a Gas Chromatography (GC) separation followed by Mass Spectroscopy (MS) detection. The purpose of the GC step is to separate multiple compounds in a sample so that they reach the MS detector one at the time.

Mass spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. Mass spectrometry has a number of applications in organic chemistry,

they are:

1. Determining molecular mass

2 Finding out the structure of unknown substances

3. Verifying the identity and purity of a known substance Providing data on isotopic abundance

4. The mass spectra of the synthesized compounds were recorded in Q-Tof-Mass Spectroscopy (Q-Tof micro hybrid quadrupole time of flight mass spectrometer). **[74] [75]**

MICROBIOLOGICAL ASSAY

Microbial assays or microbiological assays is a type of bioassay and are designed to analyse the compounds or substances which have effect on micro-organisms.

Microbiological assay is defined as the *determination or estimation of concentration or potency* of an antibiotic by means of measuring and comparing the *area of zone of inhibition or turbidity produced* by test substance with that of standard over a suitable microbe under standard conditions.

So as definition says the hypothesis is that when an antibiotic is administered, there is inhibition in the growth of microbe as indicated by decrease in area of zone of microbial colony on nutrition media or decrease in turbidity due to decrease in microbial concentration.

Uses of microbial assay:

- ❖ They help to estimate concentration and potency of antibiotics. This is not always possible by other means of estimations.
- ❖ Help in determination of the best anti-biotic suitable for patient recovery. When microbe in patients phlegm or urine is examined by biosaay, the better susceptibility of microbe to the suitable anti-biotic among those available to treat can be decided for proper treatment of infected patient. This determination is possible by immune assays like ELISA test for some diseases. **[76] [77] [78]**

alamarBlue® ASSAY FOR ESTIMATING THE MICROBIAL VIABILITY[79]

Introduction

alamarBlue® is designed to provide a rapid and sensitive measure of cell proliferation and cytotoxicity in various human and animal cell lines, bacteria and fungi. It is simple to use as the indicator dye is water soluble, thus eliminating the

washing/fixing and extraction steps required in other commonly used cell proliferation assays.

The assay incorporates a specially selected oxidation-reduction (REDOX) indicator that both fluoresces and undergoes colorimetric change in response to cellular metabolic reduction. This offers the user a choice of detection method.

alamarBlue® reagent contains **Resazurin** (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide) a blue dye, which itself is non fluorescent until it is reduced to the pink colored and highly red fluorescent **resorufin**. It is used mainly as anoxidation-reduction indicator in cell viability assays for bacteria and mammalian cells. Usually it is available commercially as the sodium salt.

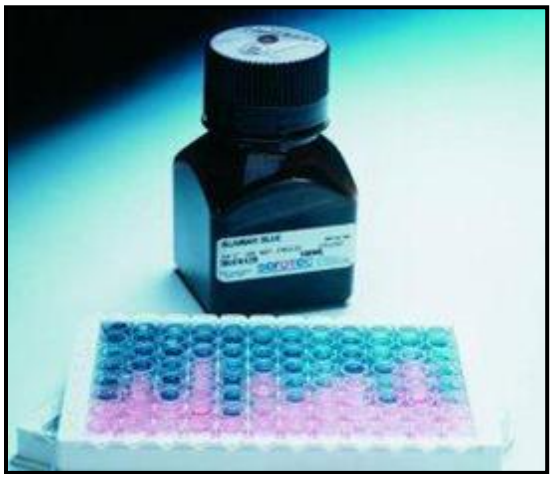


Fig 08- alamarBlue Reagent

ASSAY PROTOCOL FOR ESTIMATING ANTI-TB ACTIVITY USING alamarBlue® DYE

1) The anti mycobacterial activity of compounds were assessed against M. tuberculosis using alamarBlue® micro plate assay (MABA).

2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

3) Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 µg/ml.

6) Plates were covered and sealed with paraffin and incubated at 37ºC for five days.

7) After this time, 25µl of freshly prepared 1:1 mixture of alamarBlue® reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

FEATURES AND BENEFITS

FEATURES

- Fluorescent/colorimetric reaction
- Water soluble
- Works on suspended or attached cell lines
- Fewer steps than traditional assays
- Easily adaptable to automation
- Stable
- Non-cytotoxic
- Non-radioactive

BENEFITS

- Allows choice of detection method
- No extraction required
- No centrifugation required
- Time saving
- Useful for high throughput testing
- Allows for continuous cell growth monitoring and kinetic studies over several days
- Minimal interference with
- Normal metabolism
- Safe and easily disposable

5. RESULTS AND DISCUSSION

RESULTS OF DRUG DESIGN

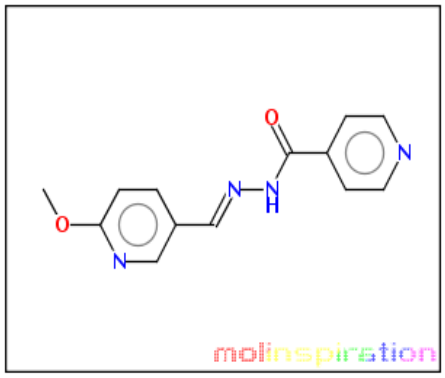
To predict the possible binding modes and enzyme inhibition mechanism, compounds were docked onto the active sites of DAPDC (LysA), using Argus Lab software 4.0. The best pose was selected based on good G-Score and the favorable interactions formed between the compound and amino acid residues of the LysA active site. All the ligands in the complex structures showed the hydrogen bond interactions with **LYS 407, SER 49, SER 361, ARG 135, TYR 302.** This clearly indicates that these hydrogen bonded amino acids play a crucial role in LysA inhibition activity.

A. DRUG LIKENESS

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A druglike molecule has properties such as: hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and course presence of various pharmacophoric features influence the the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.

INSILICO DRUG LIKENESS PREDICTION

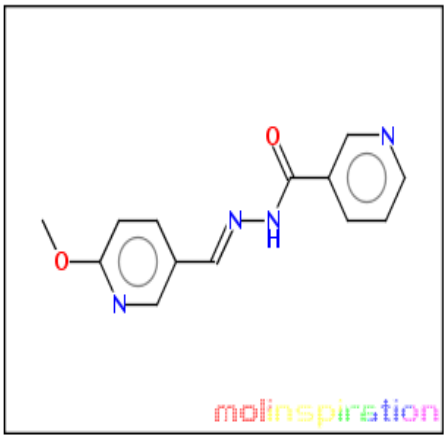
RK1



Molinspiration property engine v2014.11

miLogP	0.97
TPSA	76.48
natoms	19
MW	256.26
nON	6
nOHNH	1
nviolations	0
nrotb	4
volume	227.33

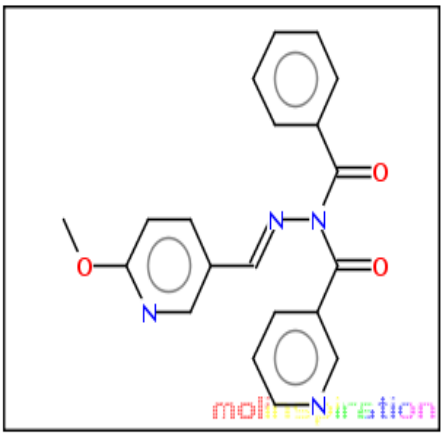
RK2



Molinspiration property engine v2014.11

miLogP	1.02
TPSA	76.48
natoms	19
MW	256.26
nON	6
nOHNH	1
nviolations	0
nrotb	4
volume	227.33

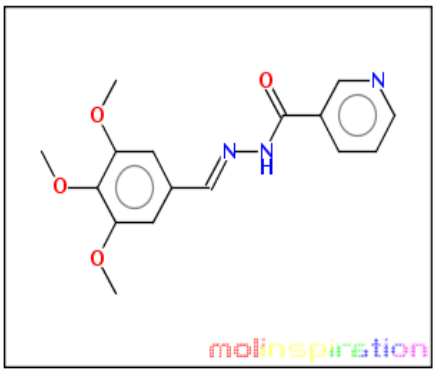
RK2a



Molinspiration property engine v2014.11

miLogP	1.77
TPSA	84.76
natoms	27
MW	360.37
nON	7
nOHNH	0
nviolations	0
nrotb	5
volume	318.11

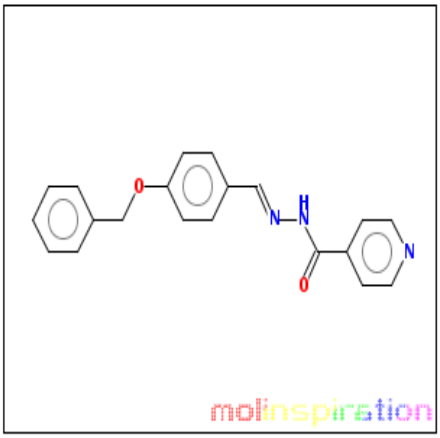
RK3



Molinspiration property engine v2014.11

miLogP	1.49
TPSA	82.06
natoms	23
MW	315.33
nON	7
nOHNH	1
nviolations	0
nrotb	6
volume	282.58

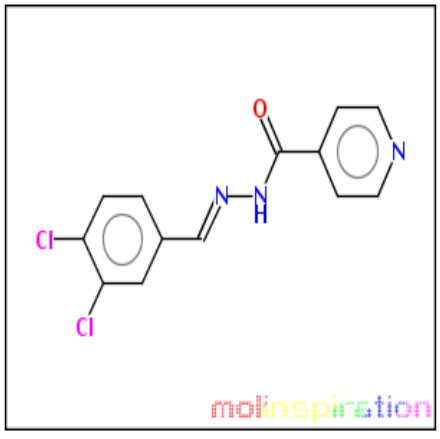
RK4



Molinspiration property engine v2014.11

miLogP	3.46
TPSA	63.59
natoms	25
MW	331.38
nON	5
nOHNH	1
nviolations	0
nrotb	6
volume	303.14

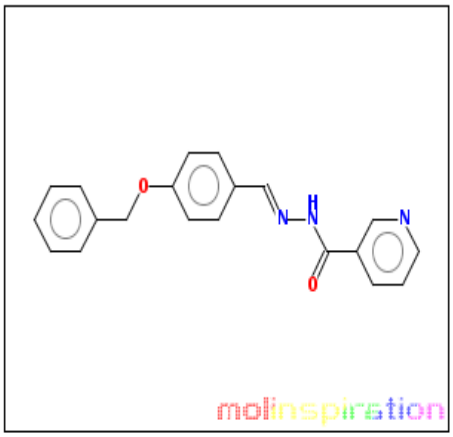
RK5



Molinspiration property engine v2014.11

miLogP	3.09
TPSA	54.35
natoms	19
MW	294.14
nON	4
nOHNH	1
nviolations	0
nrotb	3
volume	233.01

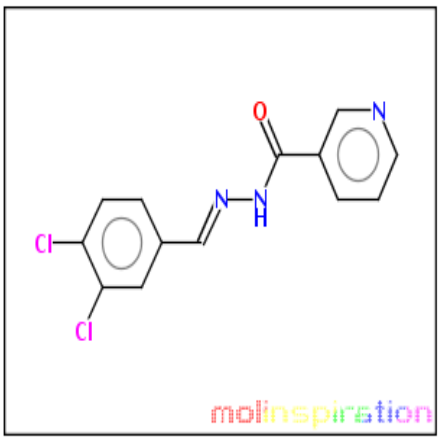
RK6



Molinspiration property engine v2014.11

miLogP	3.51
TPSA	63.59
natoms	25
MW	331.38
nON	5
nOHNH	1
nviolations	0
nrotb	6
volume	303.14

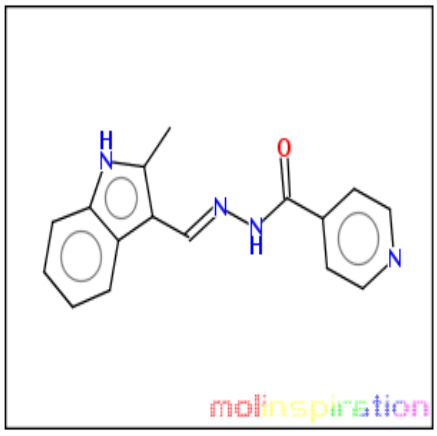
RK7



Molinspiration property engine v2014.11

miLogP	3.15
TPSA	54.35
natoms	19
MW	294.14
nON	4
nOHNH	1
nviolations	0
nrotb	3
volume	233.01

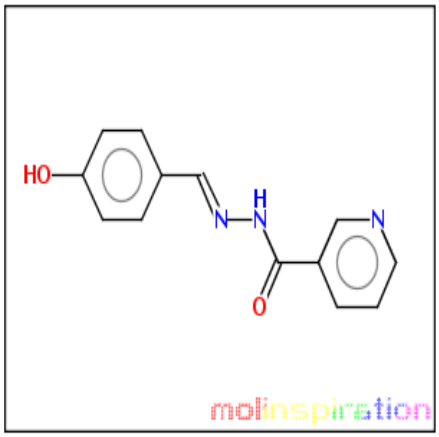
RK8



Molinspiration property engine v2014.11

miLogP	2.18
TPSA	70.14
natoms	21
MW	278.31
nON	5
nOHNH	2
nviolations	0
nrotb	3
volume	251.48

RK9



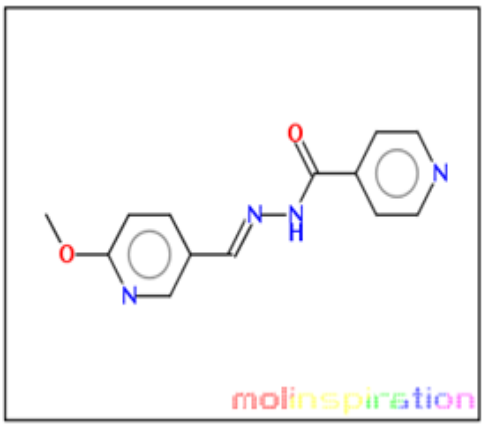
Molinspiration property engine v2014.11

miLogP	1.38
TPSA	74.58
natoms	18
MW	241.25
nON	5
nOHNH	2
nviolations	0
nrotb	3
volume	213.96

INSILICO DRUG BIOACTIVITY PREDICTION

Prediction of bioactivity score for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors.

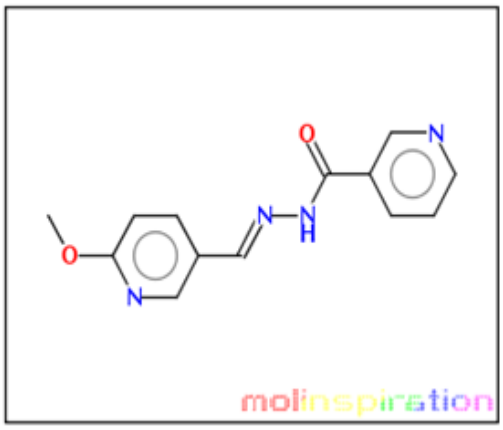
RK1



Molinspiration bioactivity score

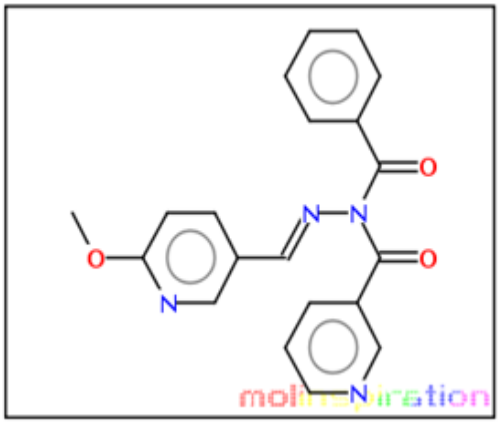
GPCR ligand	-0.33
Ion channel modulator	-0.70
Kinase inhibitor	-0.36
Nuclear receptor ligand	-0.71
Protease inhibitor	-0.71
Enzyme inhibitor	-0.20

RK2



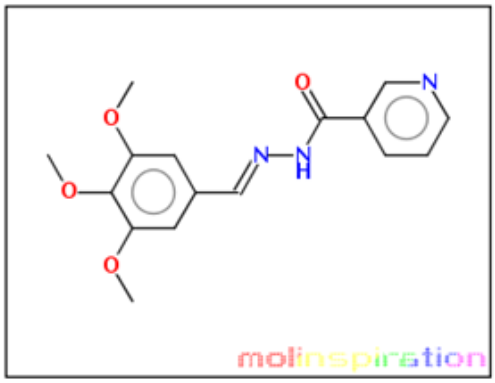
Molinspiration bioactivity score	
GPCR ligand	-0.31
Ion channel modulator	-0.68
Kinase inhibitor	-0.38
Nuclear receptor ligand	-0.73
Protease inhibitor	-0.71
Enzyme inhibitor	-0.19

RK2a



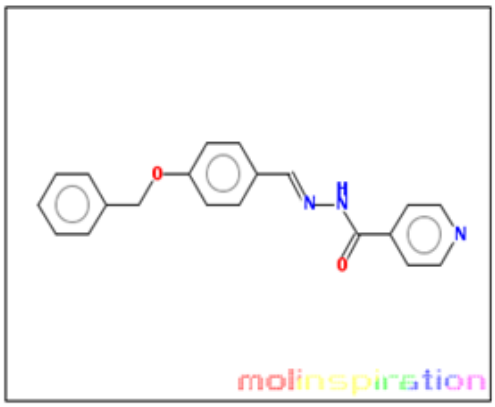
Molinspiration bioactivity score	
GPCR ligand	-0.11
Ion channel modulator	-0.30
Kinase inhibitor	-0.07
Nuclear receptor ligand	-0.33
Protease inhibitor	-0.30
Enzyme inhibitor	-0.01

RK3



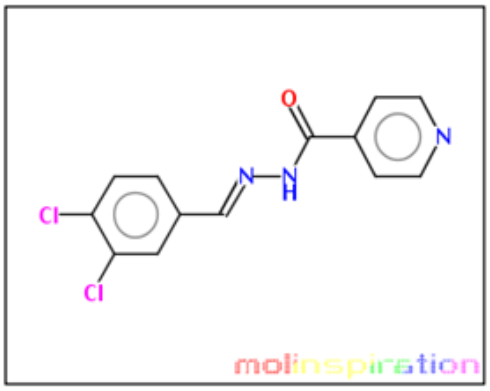
Molinspiration bioactivity score	
GPCR ligand	-0.34
Ion channel modulator	-0.72
Kinase inhibitor	-0.28
Nuclear receptor ligand	-0.66
Protease inhibitor	-0.58
Enzyme inhibitor	-0.34

RK4



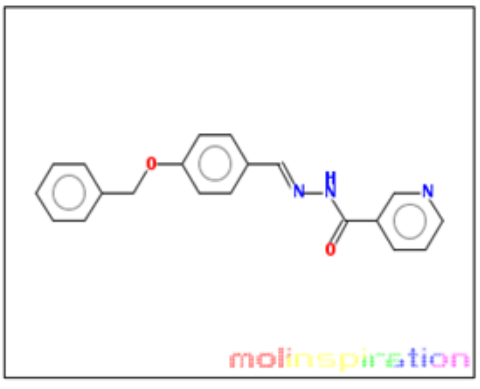
Molinspiration bioactivity score	
GPCR ligand	-0.24
Ion channel modulator	-0.59
Kinase inhibitor	-0.23
Nuclear receptor ligand	-0.40
Protease inhibitor	-0.34
Enzyme inhibitor	-0.26

RK5



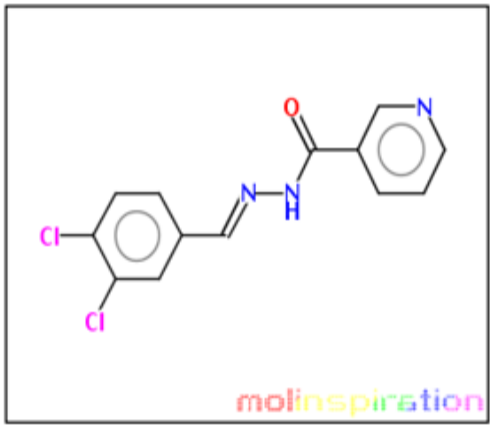
Molinspiration bioactivity score	
GPCR ligand	-0.46
Ion channel modulator	-0.75
Kinase inhibitor	-0.46
Nuclear receptor ligand	-0.82
Protease inhibitor	-0.78
Enzyme inhibitor	-0.43

RK6



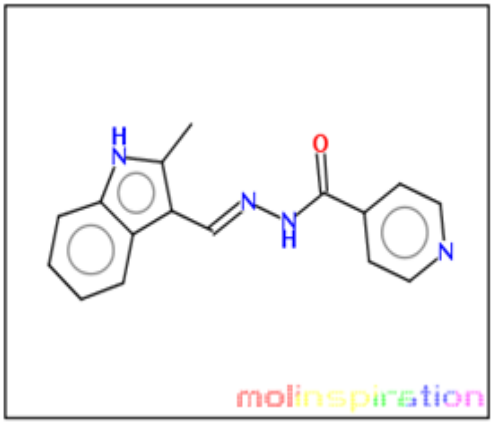
Molinspiration bioactivity score	
GPCR ligand	-0.22
Ion channel modulator	-0.56
Kinase inhibitor	-0.23
Nuclear receptor ligand	-0.41
Protease inhibitor	-0.34
Enzyme inhibitor	-0.24

RK7



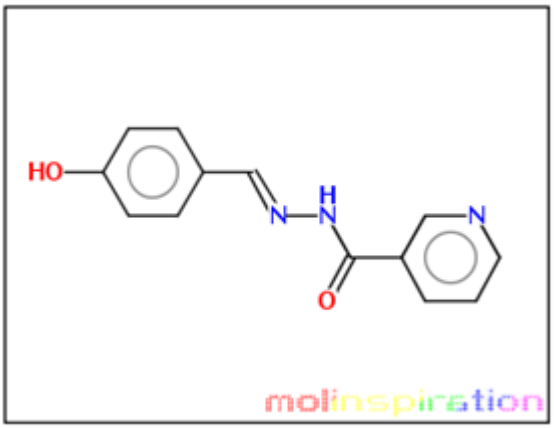
Molinspiration bioactivity score	
GPCR ligand	-0.43
Ion channel modulator	-0.72
Kinase inhibitor	-0.45
Nuclear receptor ligand	-0.83
Protease inhibitor	-0.78
Enzyme inhibitor	-0.40

RK8



Molinspiration bioactivity score	
GPCR ligand	-0.29
Ion channel modulator	-0.67
Kinase inhibitor	-0.21
Nuclear receptor ligand	-0.69
Protease inhibitor	-0.65
Enzyme inhibitor	-0.33

RK9

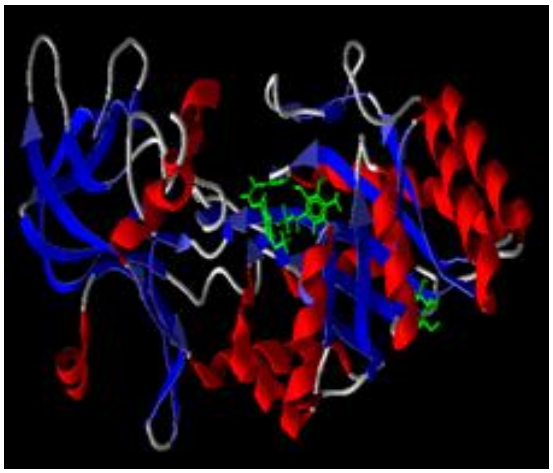


Molinspiration bioactivity score	
GPCR ligand	-0.46
Ion channel modulator	-0.72
Kinase inhibitor	-0.44
Nuclear receptor ligand	-0.71
Protease inhibitor	-0.80
Enzyme inhibitor	-0.32

RESULTS OF DOCKING REPORTS

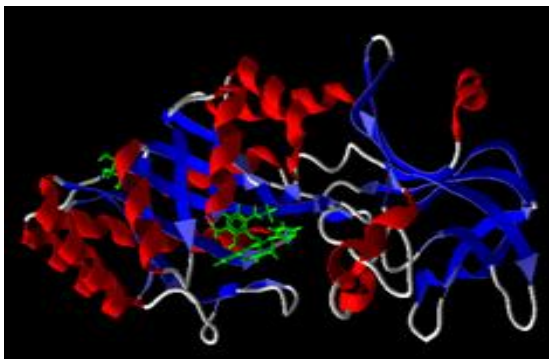
The designed molecules were docked against the selected target Diaminopimelate decarboxylase (LysA) extra precision mode. Molecular docking was executed for perfect docking of the ligand into the cavity of the protein having active site .During the docking procedure different poses of the ligand were generated and the ligands were docked in different poses .The best docked poses were selected based on the energy generated and the interactions between the protein and the ligand. There were hydrogen bond interactions between all the ten molecules and the active site. Hydrophobic interactions were also observed.

DOCKING SCORE OF THE COMPOUNDS

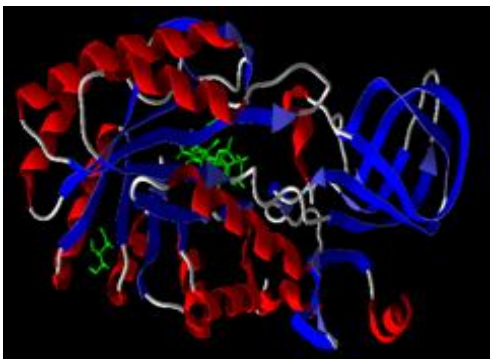


RK 1-Score:-7.14184 Kcal/Mol

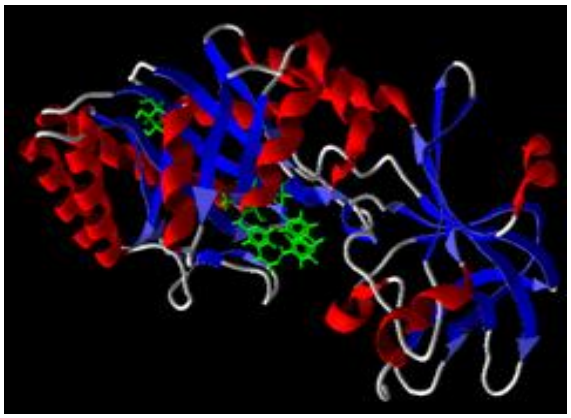
RK 2-Score:-6.0990 Kcal/Mol



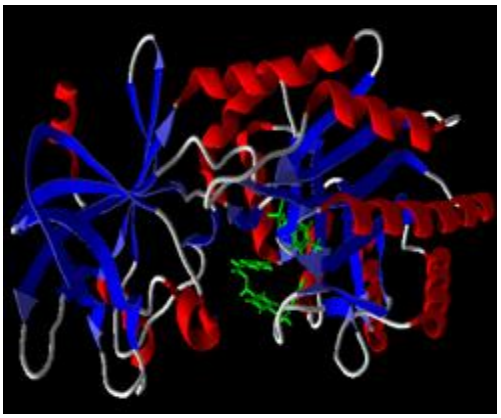
RK 2a-Score:-7.01767 Kcal/Mol



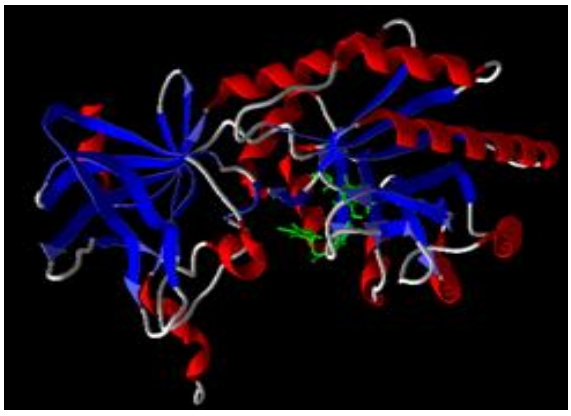
RK 3-Score:-7.02594 Kcal/Mol



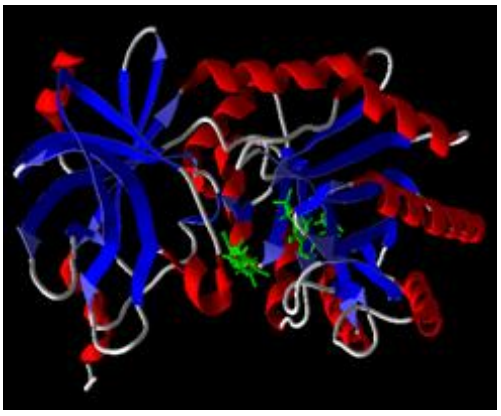
RK 4-Score:-7.00587 Kcal/Mol



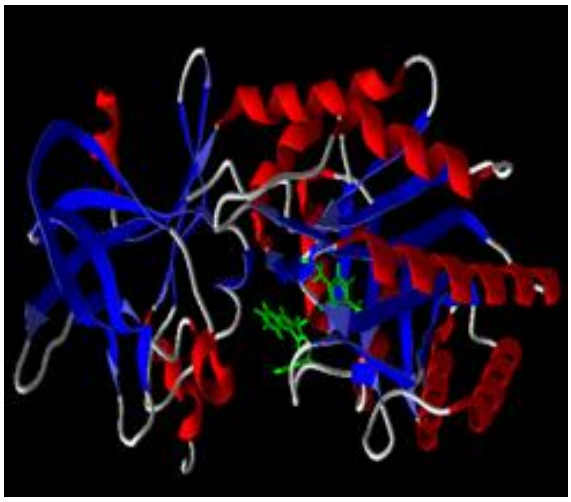
RK 5-Score:-8.86707 Kcal/Mol



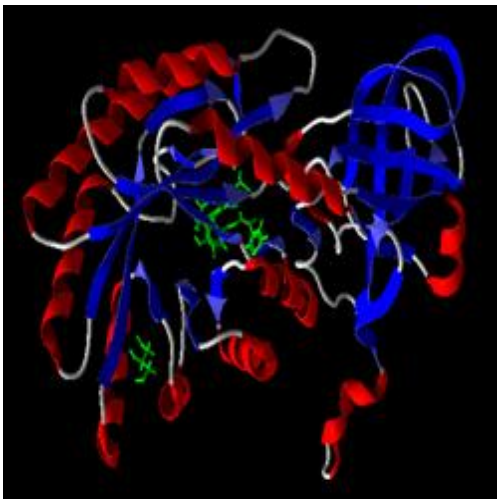
RK 6-Score:-7.93265 Kcal/Mol



RK 7-Score:-8.32154 Kcal/Mol

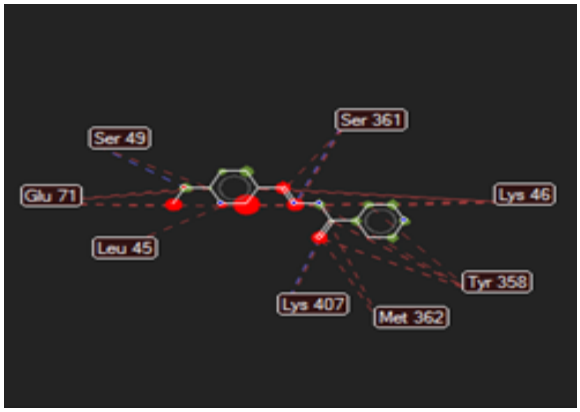


RK 8-Score:-9.43975 Kcal/Mol

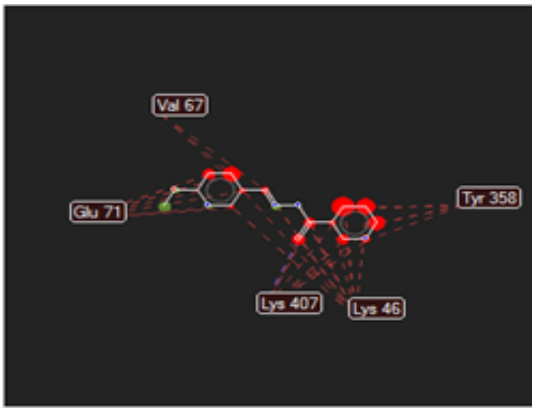


RK 9-Score:-8.13855 Kcal/Mol

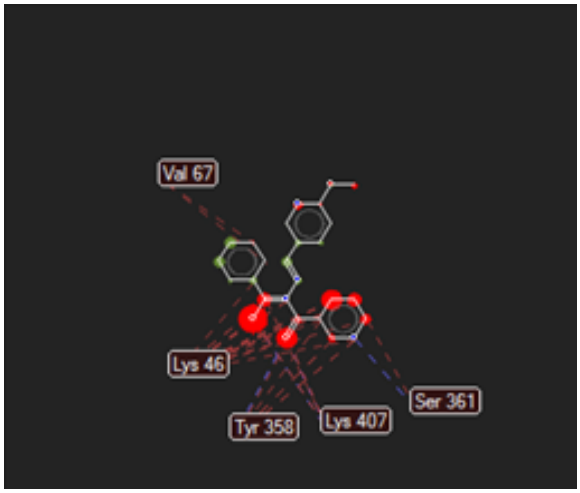
LIGAND INTERACTION REPORTS



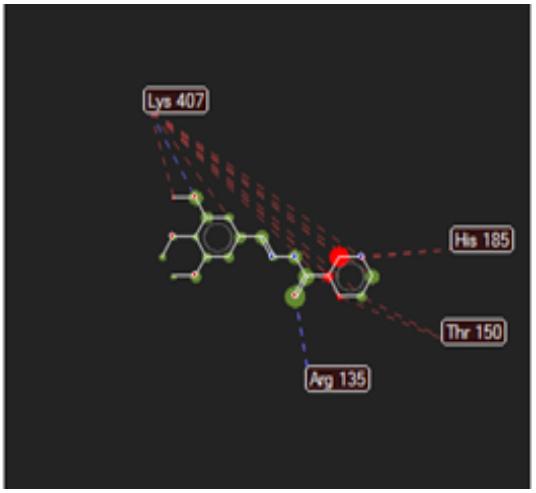
RK1



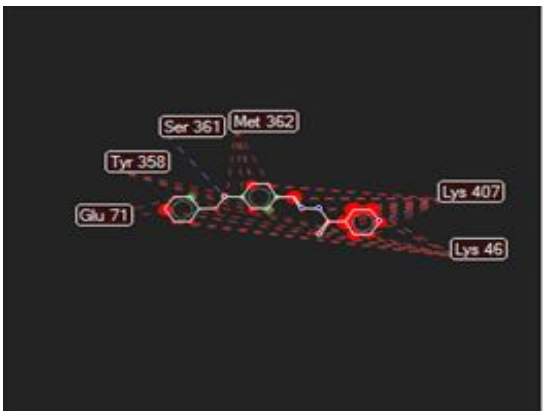
RK2



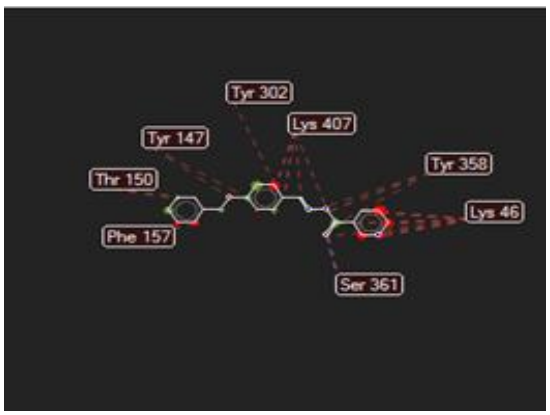
RK2a



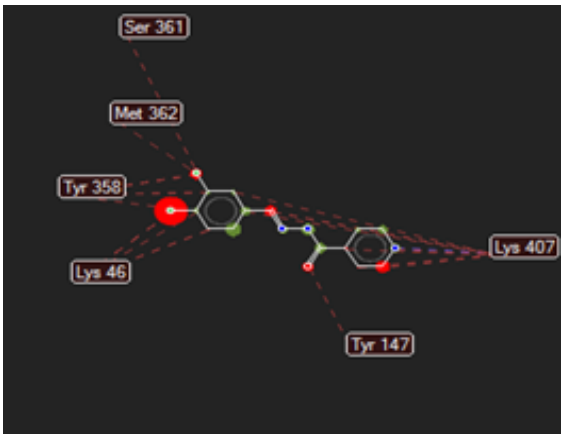
RK3



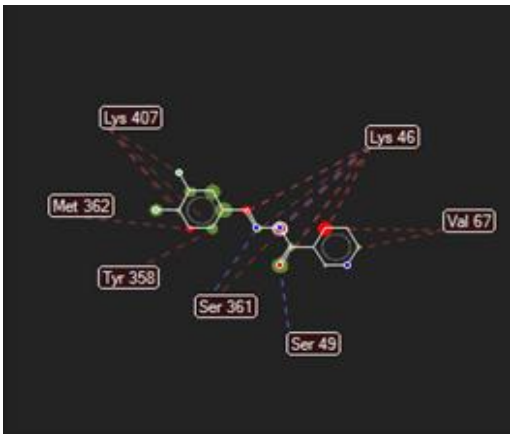
RK4



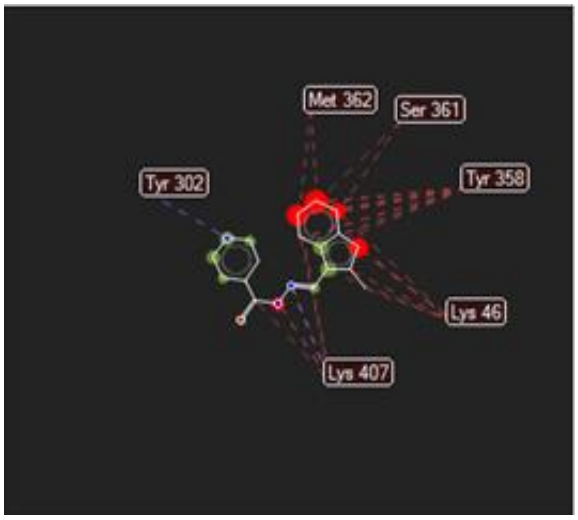
RK5



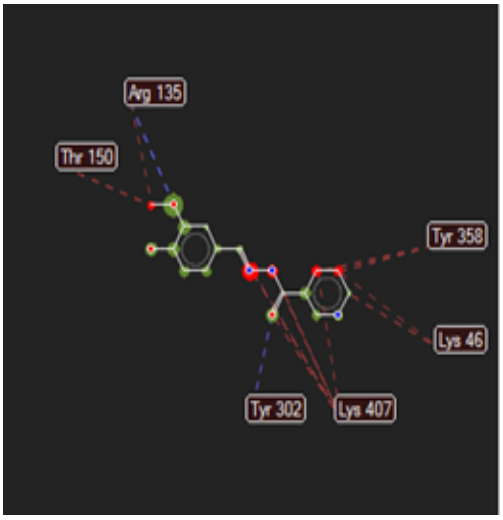
RK6



RK7



RK8



RK9

The designed molecules were also docked against some other targets of Mycobacterium Tuberculosis such as

1) Alpha 1,4-N- Acetyl glycosaminyl transferase **(PDB ID- 4EEG) -** Arginine biosynthesis for regulates cell wall and cell processes.

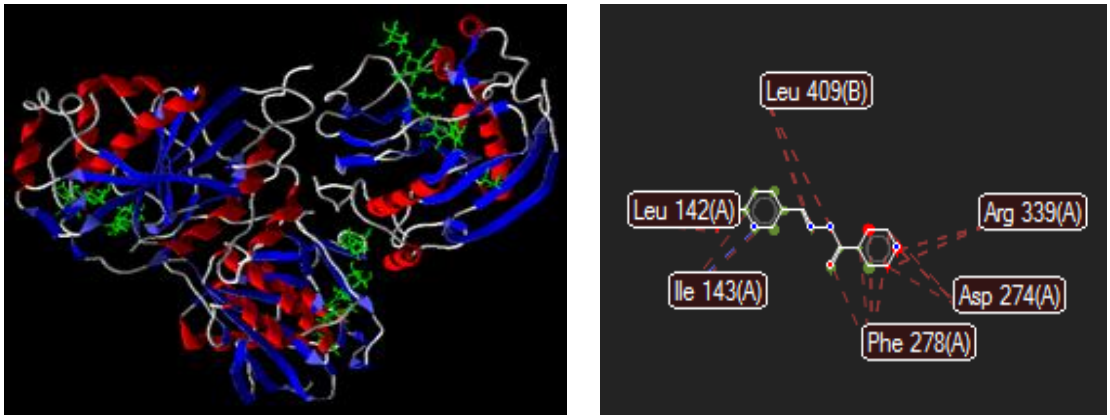
2) D-3 Phosphoglycerate dehydrogenase **(PDB ID- 1YGY) –** 6-Serine biosynthesis for cell signaling.

3) Pyridoxamine 5-phosphate oxidase **(PDB ID-5BNC) –** Pyridoxine synthesis for intermediatory metabolism and respiration.

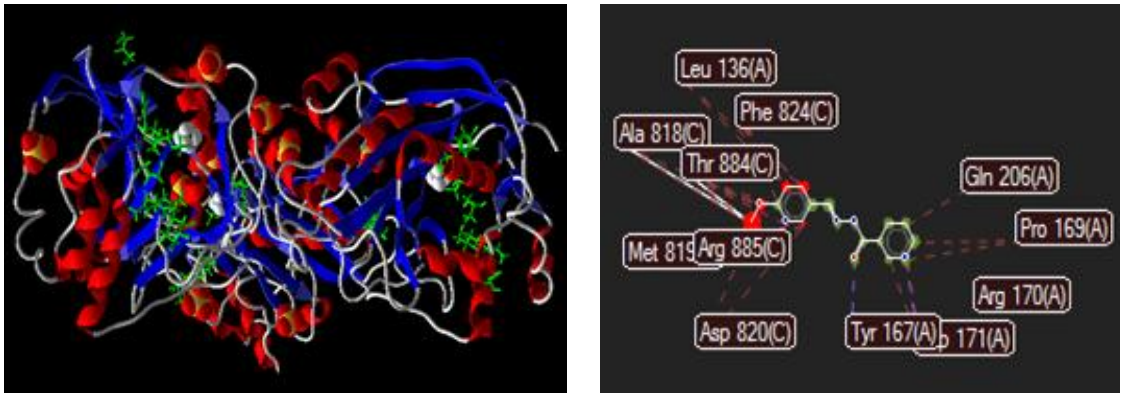
4) D-Alanyl D-Alanine Carboxypeptidase **(PDB ID- 4MPH) –** Peptidoglycan synthesis for cell wall and cell processes.

The synthesised compounds were docked with above targets showed a good G-Score and Good ligant- interaction docked with as same as the DAPDC.

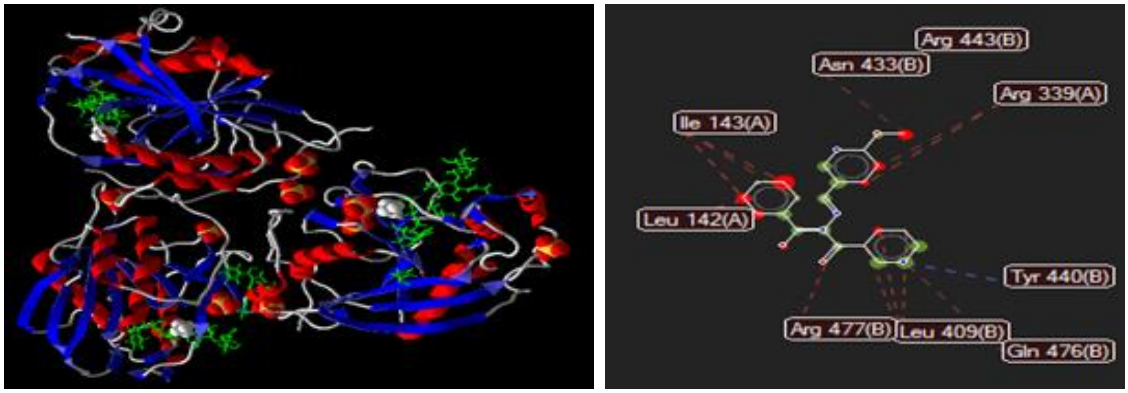
1. Docking reports and ligant interaction of syntesised copounds with Alpha 1,4-N- Acetyl glycosaminyl transferase



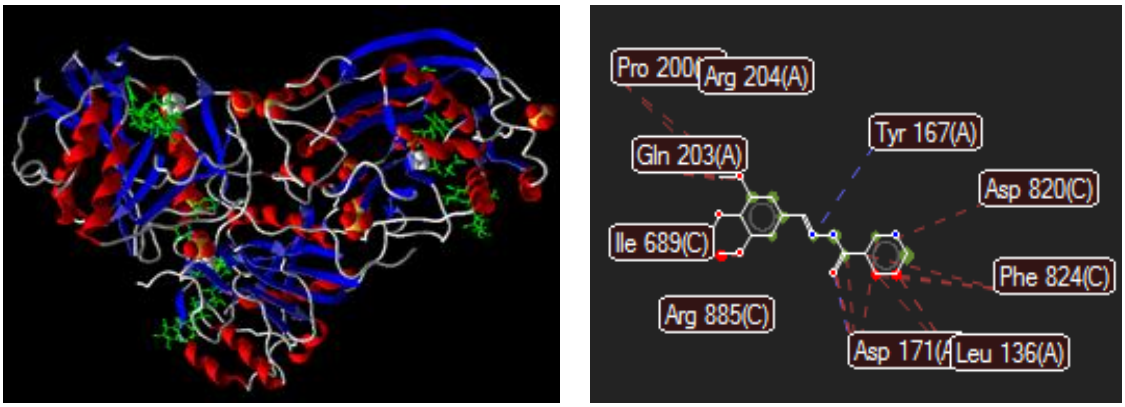
RK 1-Score:-7.49361 Kcal/Mol



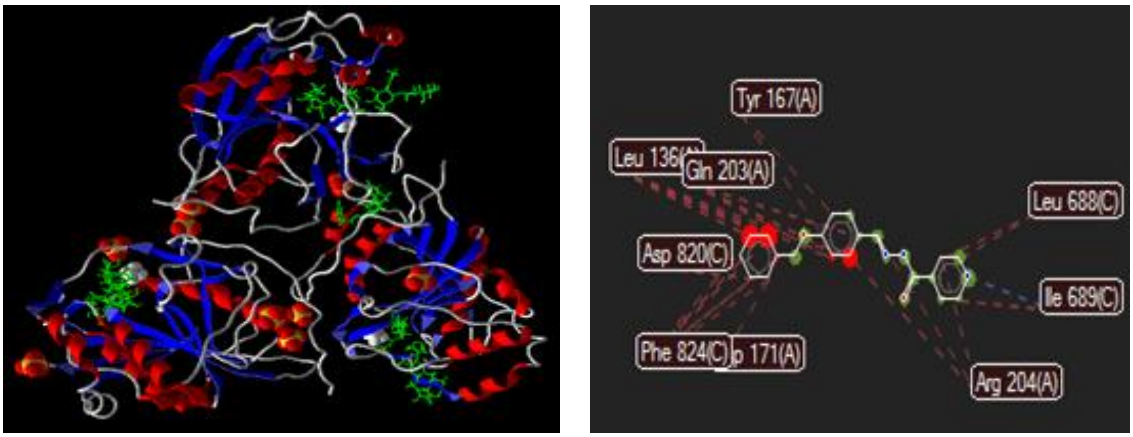
RK 2-Score:-8.35999 Kcal/Mol



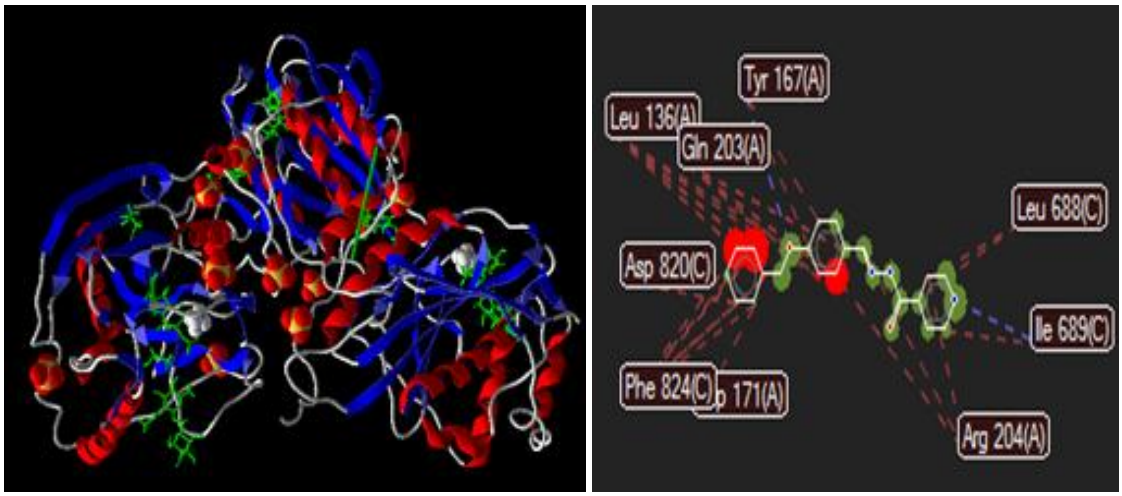
RK 2a-Score: -7.83187 Kcal/Mol



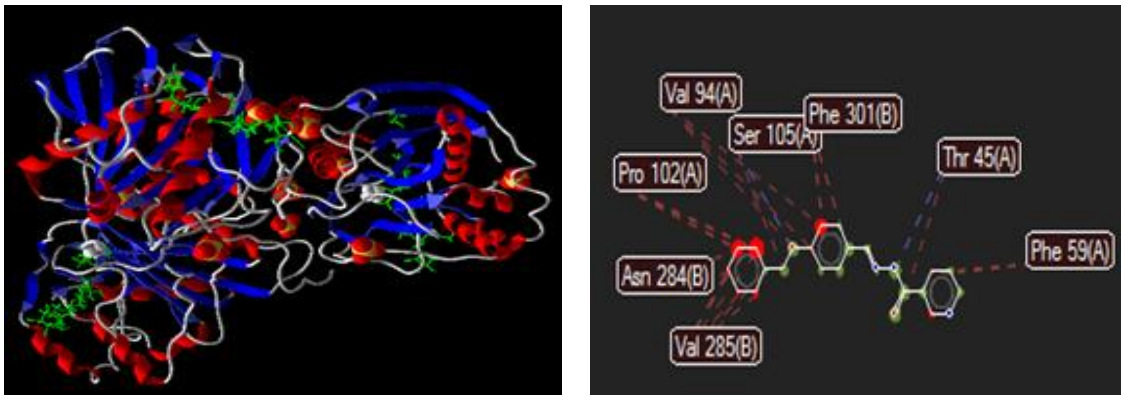
RK 3-Score:-7.94069 Kcal/Mol



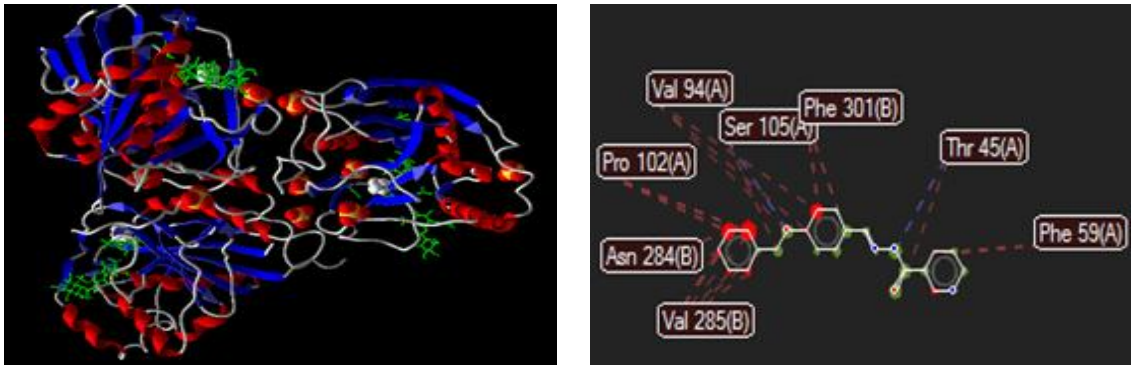
RK 4-Score:-10.0529 Kcal/Mol



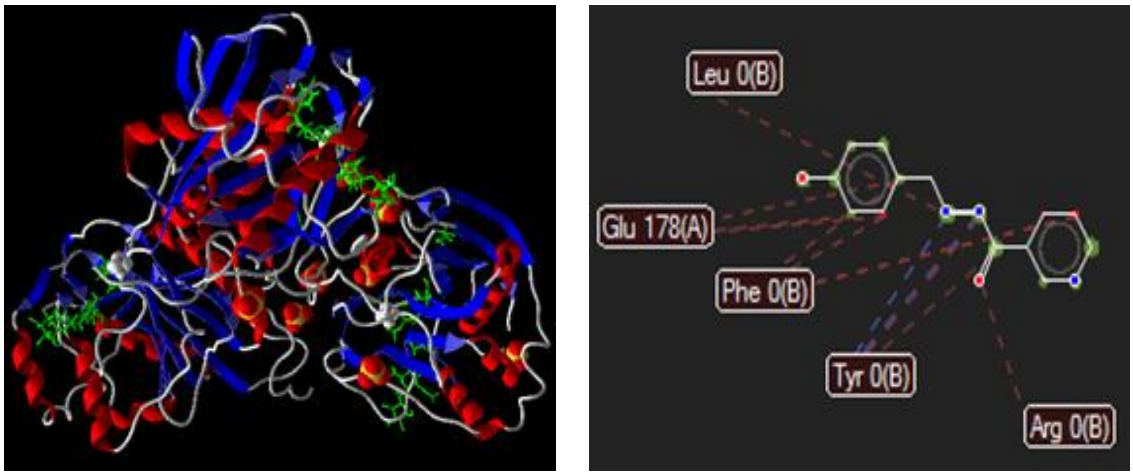
RK 5-Score:- 8.39666 Kcal/Mol



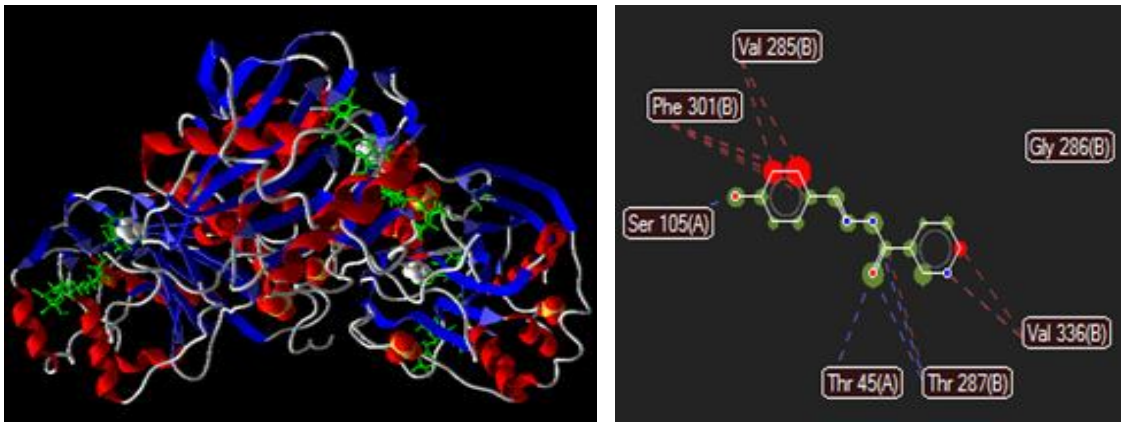
RK 6-Score:-10.033 Kcal/Mol



RK 7-Score:-8.25567 Kcal/Mol

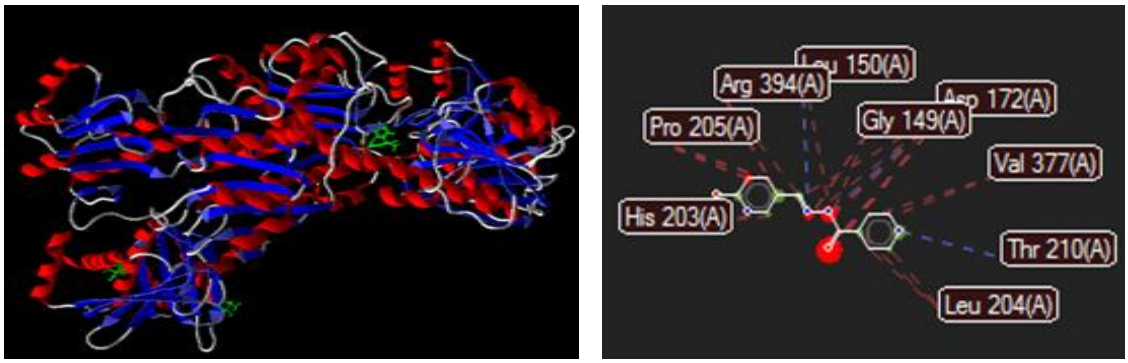


RK 8-Score:-10.2366 Kcal/Mol

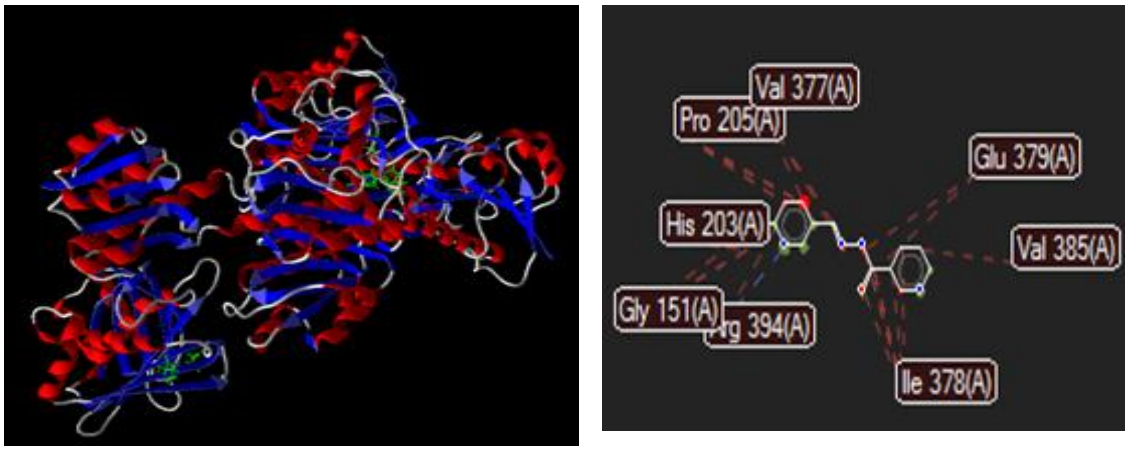


RK 9-Score:-8.00789 Kcal/Mol

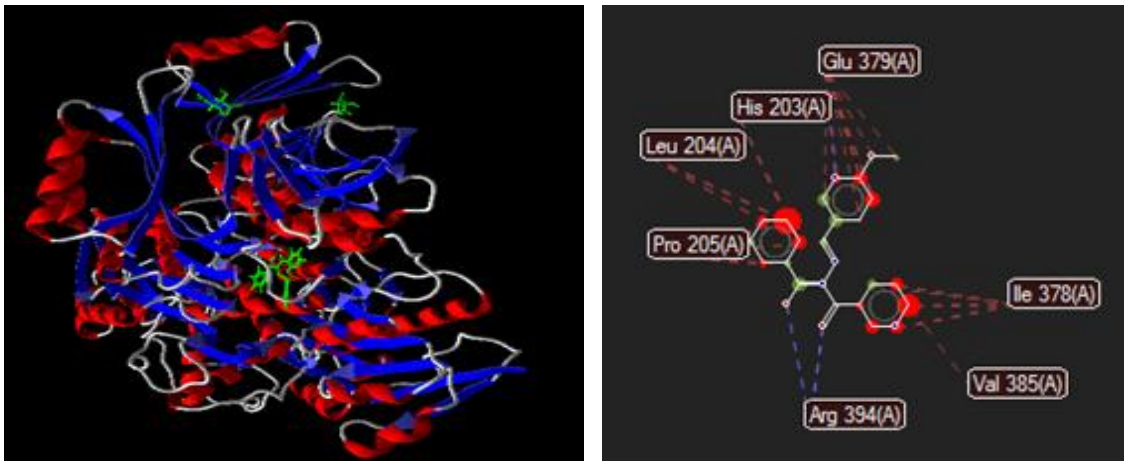
2. Docking reports and ligant interaction of syntesised copounds with D-3 Phosphoglycerate dehydrogenase



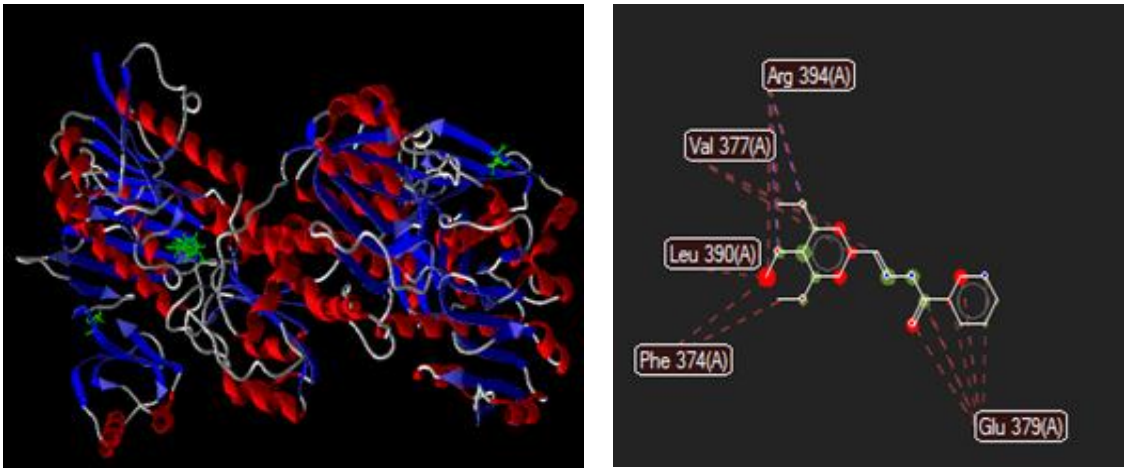
RK 1-Score:-7.1908 Kcal/Mol



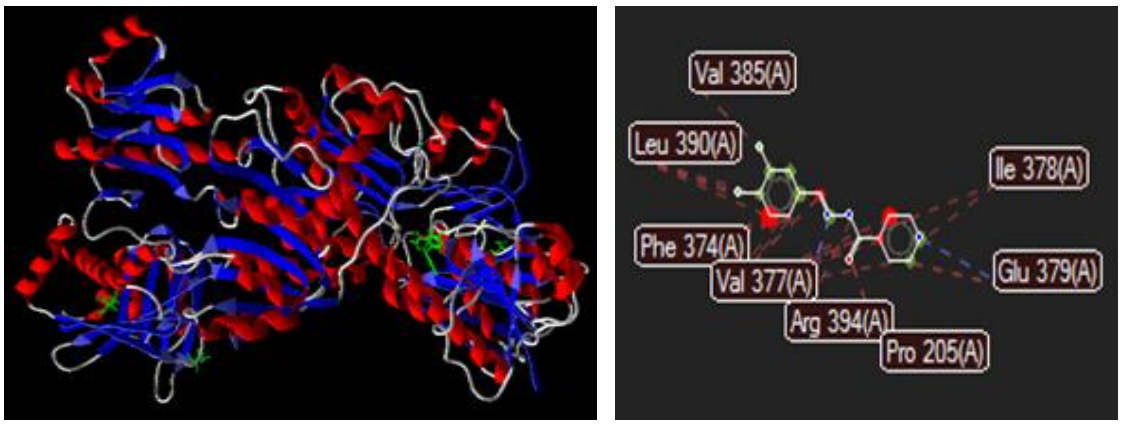
RK 2-Score:-7.67436 Kcal/Mol



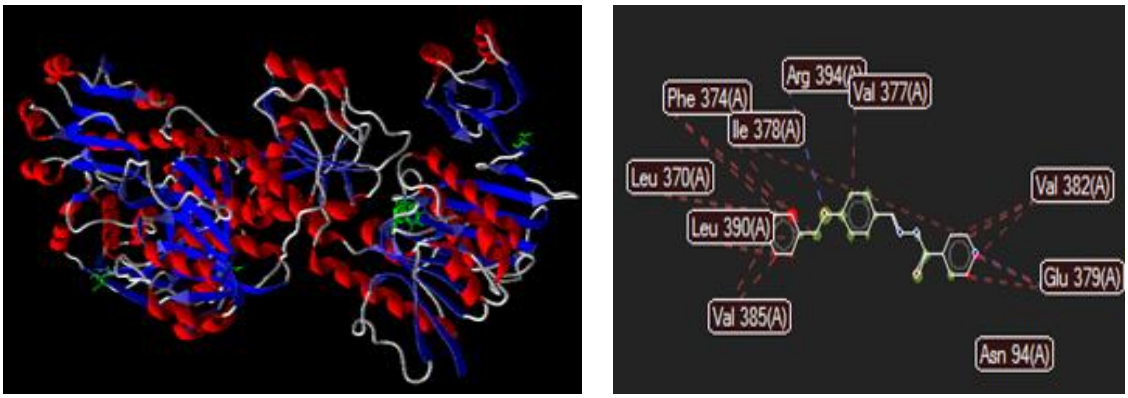
RK 2a-Score:-7.67294 Kcal/Mol



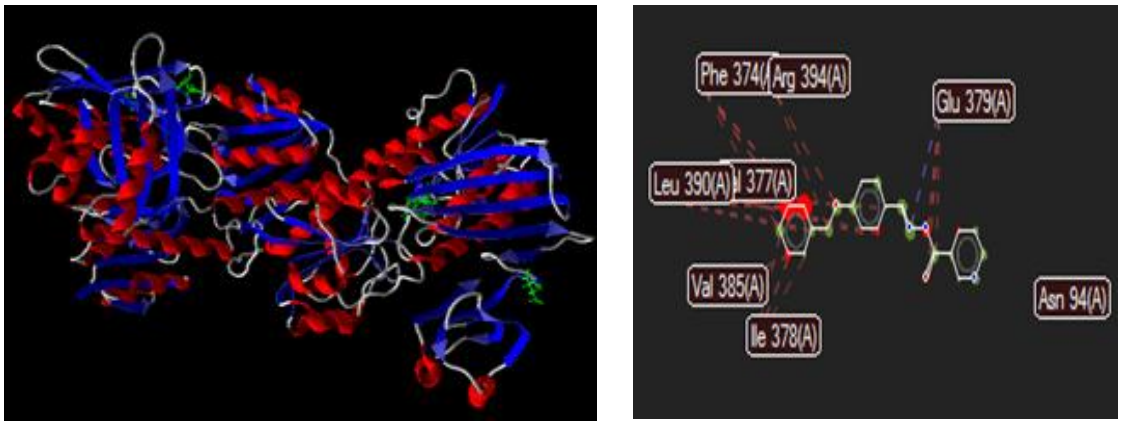
RK 3-Score:-6.55715 Kcal/Mol



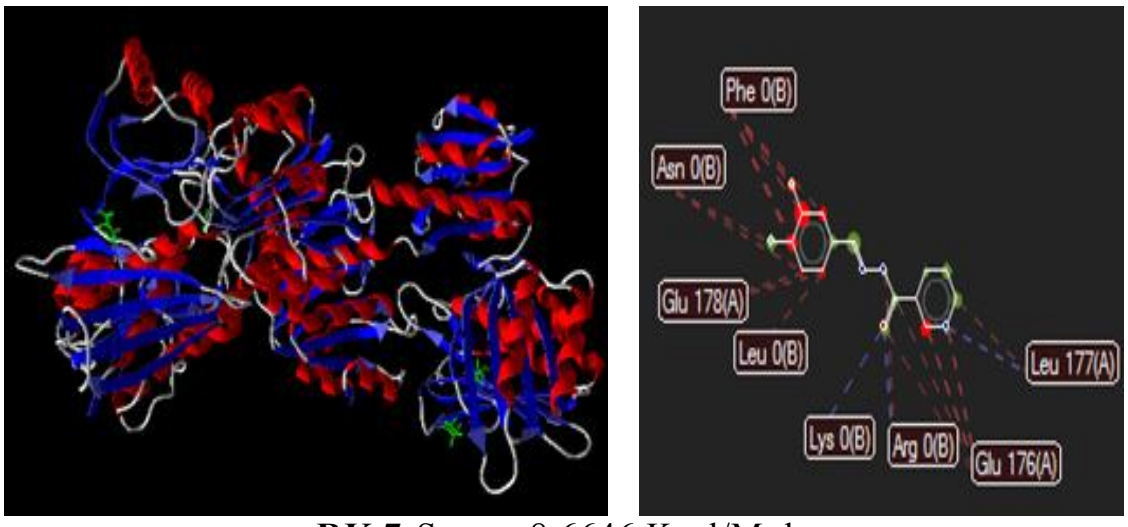
RK 4-Score:-11.1077 Kcal/Mol



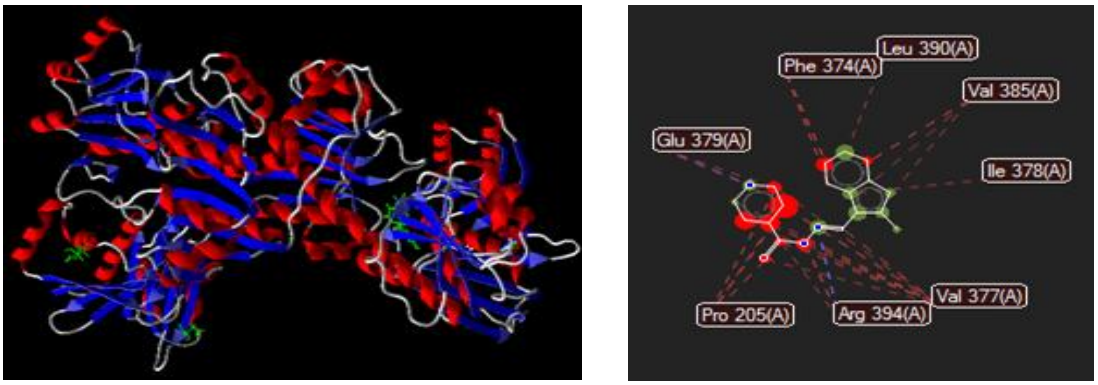
RK 5-Score:-10.7417 Kcal/Mol



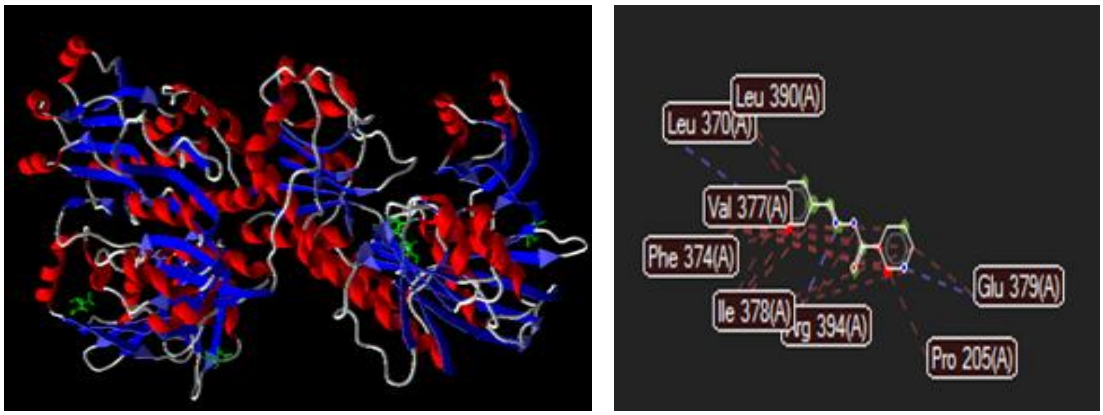
RK 6-Score:-11.4436 Kcal/Mol



RK 7-Score:-8.6646 Kcal/Mol

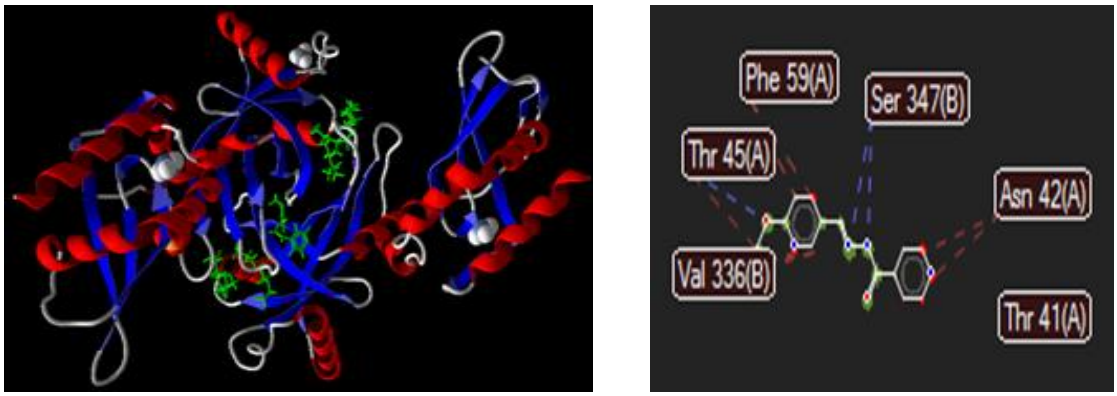


RK 8-Score:-10.5885 Kcal/Mol

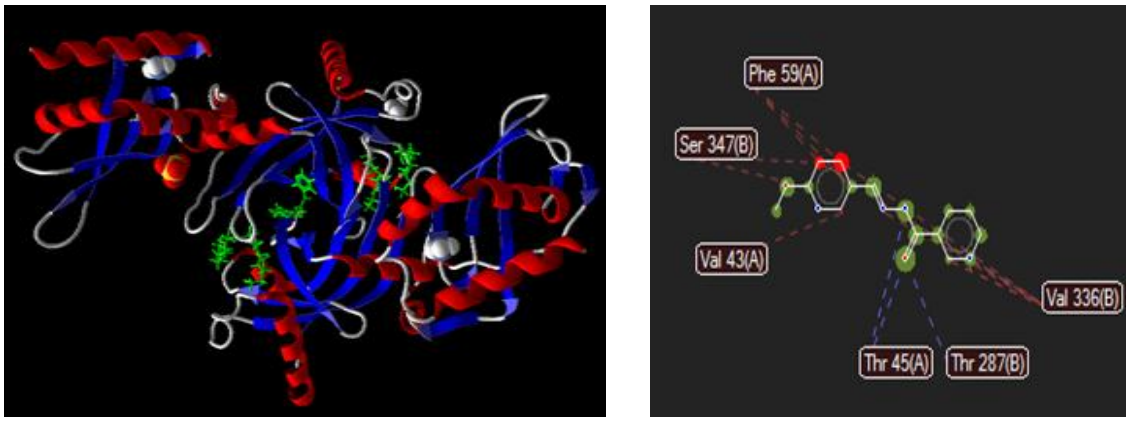


RK 9-Score:-8.63668 Kcal/Mol

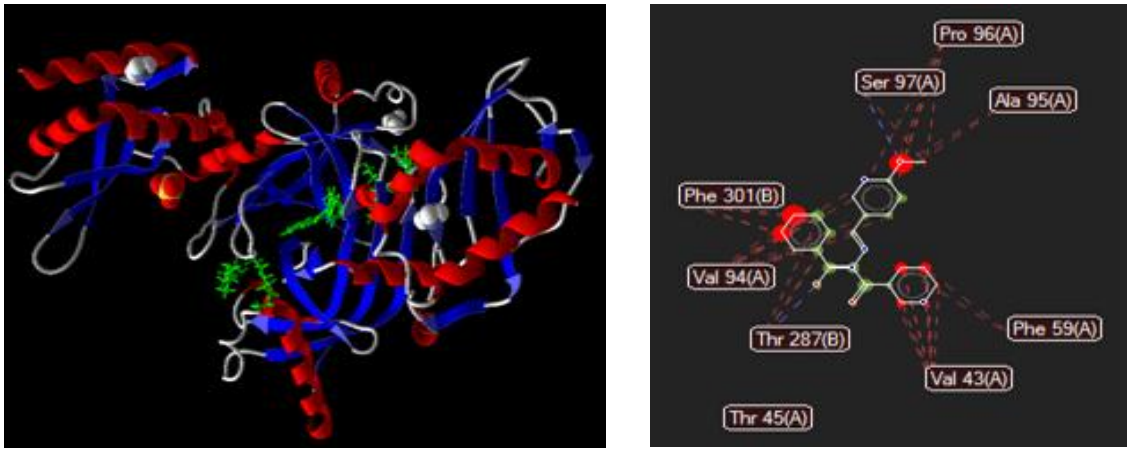
3. Docking reports and ligant interaction of syntesised copounds with Pyridoxamine 5-Phosphate oxidase



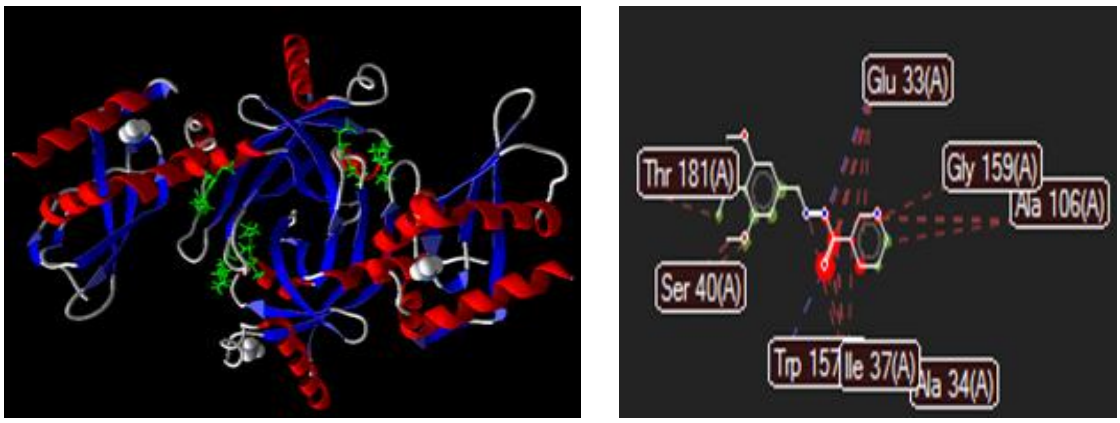
RK 1-Score:-8.03589 Kcal/Mol



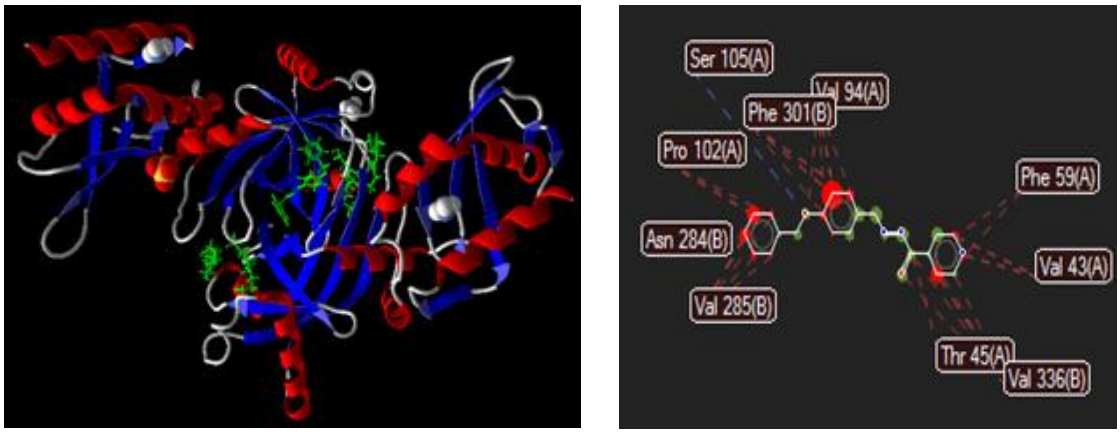
RK 2-Score:-8.23237 Kcal/Mol



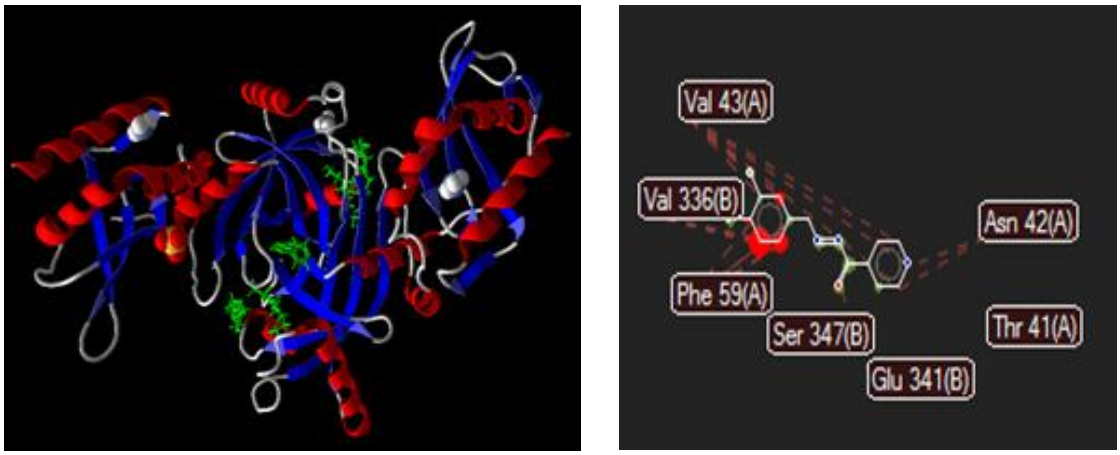
RK 2a-Score:-9.47464 Kcal/Mol



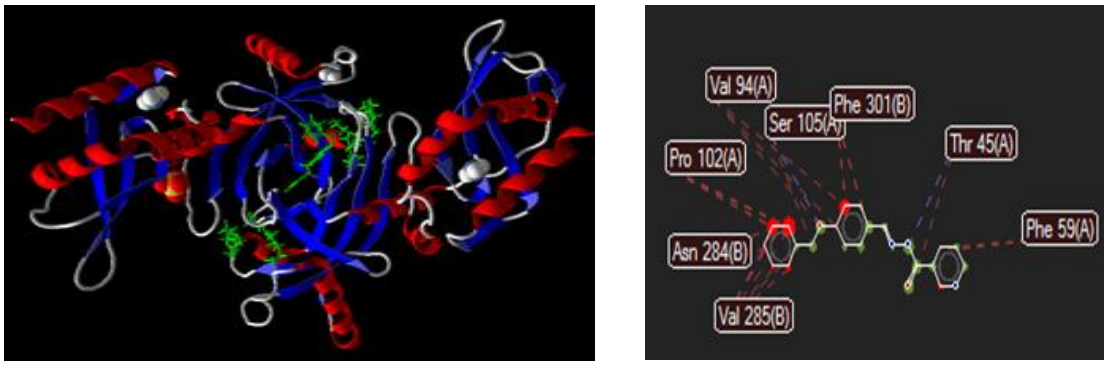
RK 3-Score:-6.45404Kcal/Mol



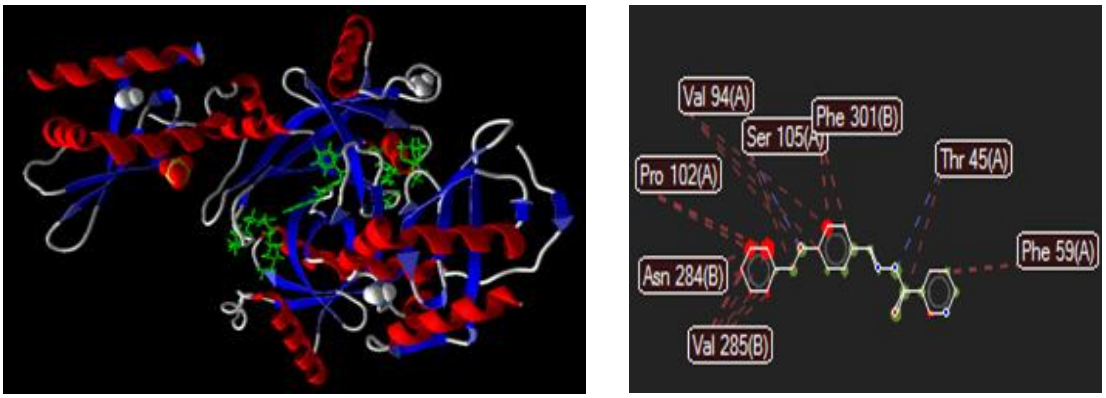
RK 4-Score:-9.63219 Kcal/Mol



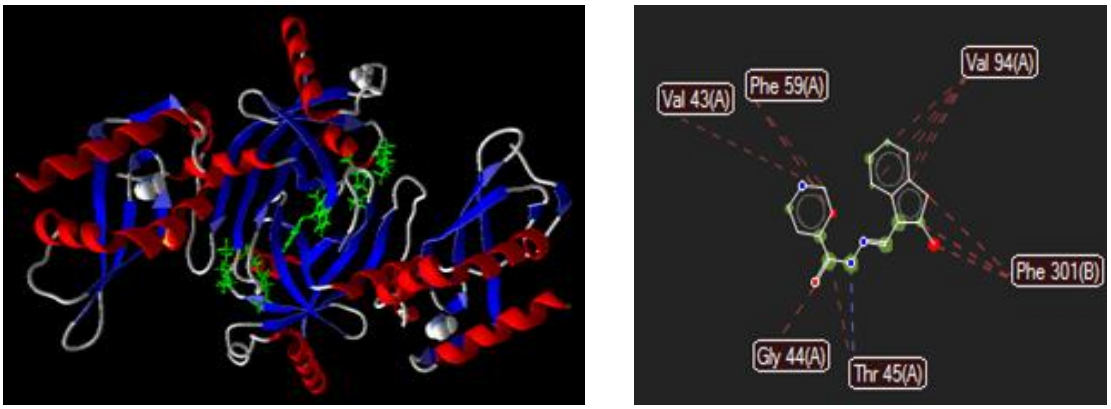
RK 5-Score:-9.5003 Kcal/Mol



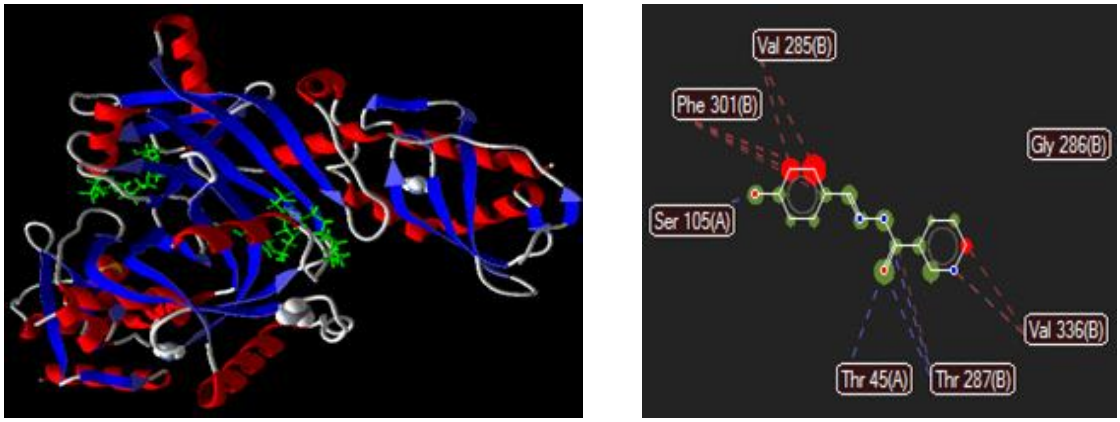
RK 6-Score:-10.1913 Kcal/Mol



RK 7-Score:-9.37594 Kcal/Mol

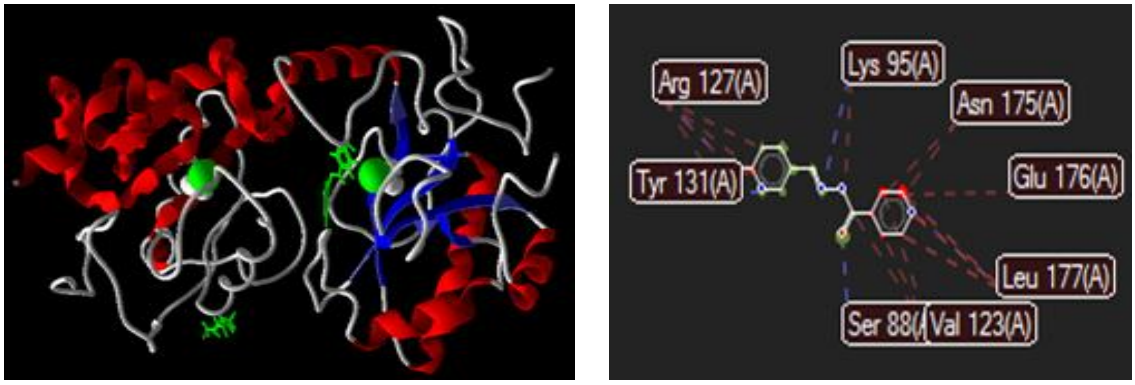


RK 8-Score:-9.60466 Kcal/Mol

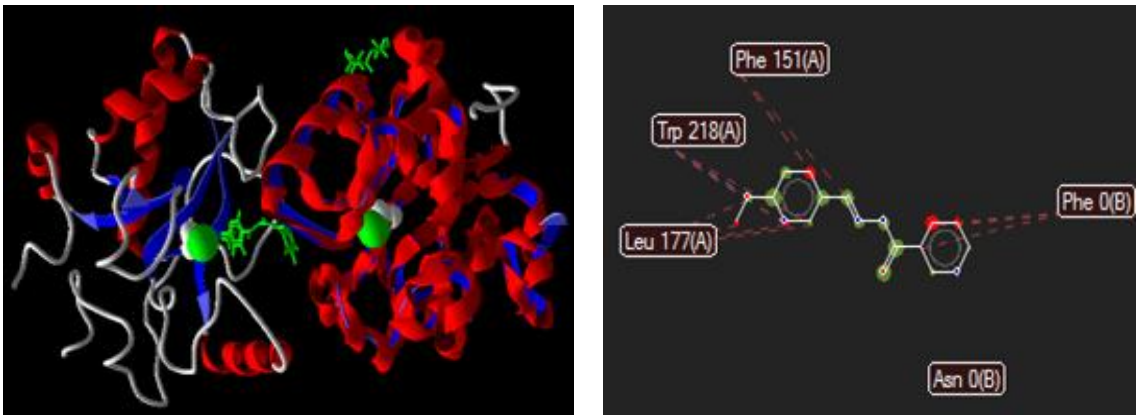


RK 9-Score:-8.4587 Kcal/Mol

4. Docking reports and ligant interaction of syntesised copounds with D-Alanyl D-Alanine carboxypeptidase

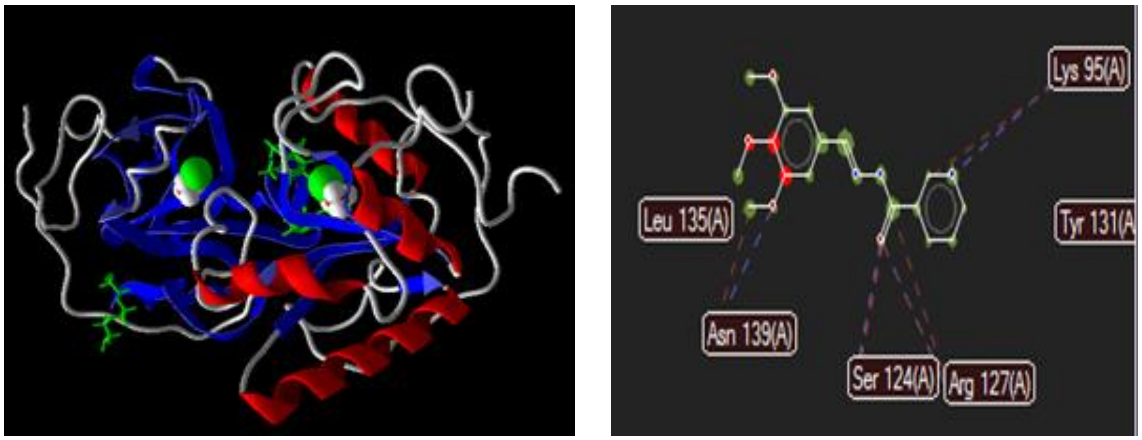


RK 1-Score:-8.01848 Kcal/Mol

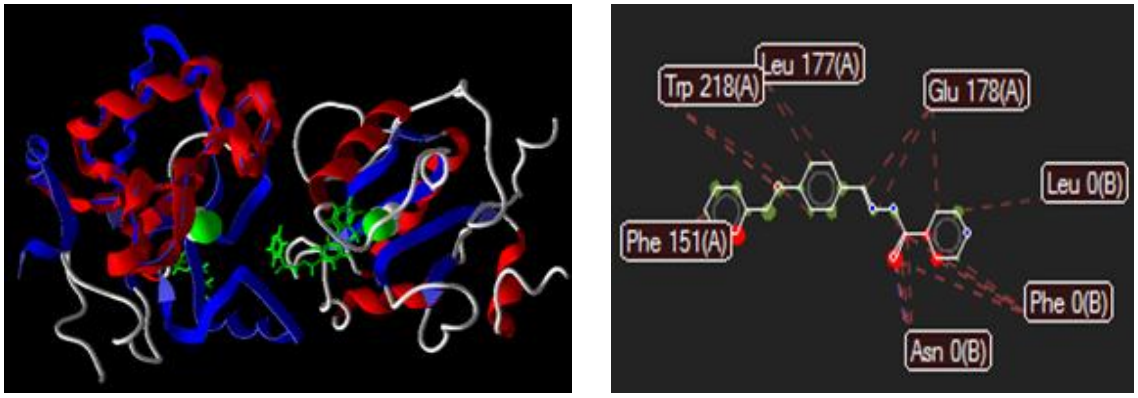


RK 2-Score:-7.20556 Kcal/Mol

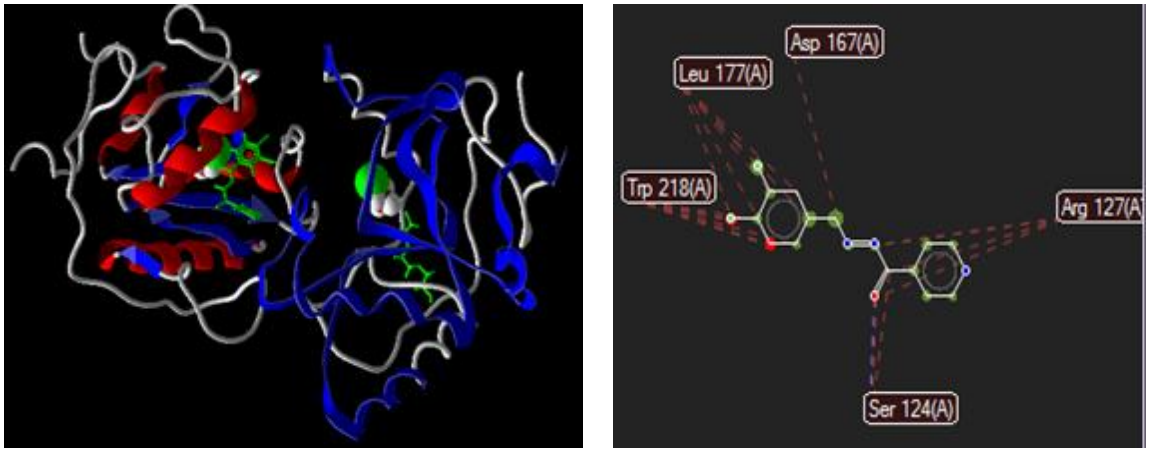
RK2a-Score:-8.63945Kcal/Mol



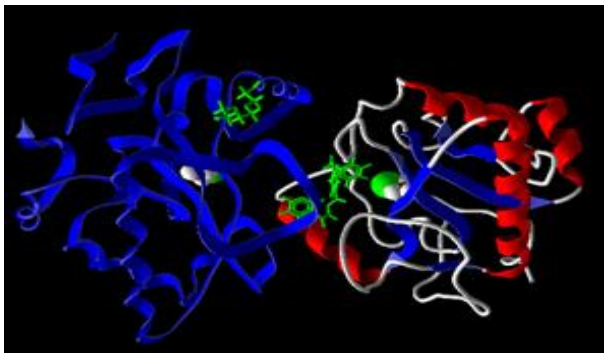
RK 3-Score:-7.78564 Kcal/Mol

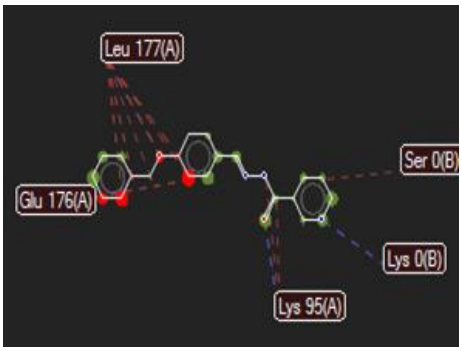


RK 4-Score:-9.6324 Kcal/Mol

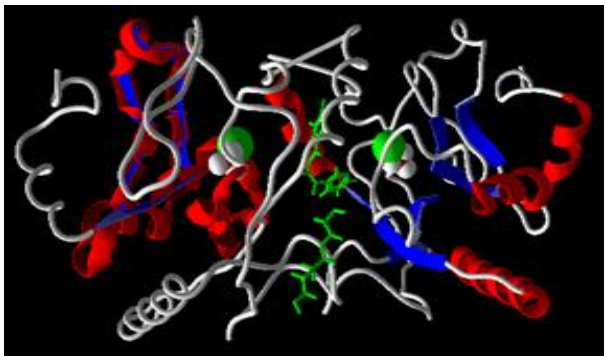


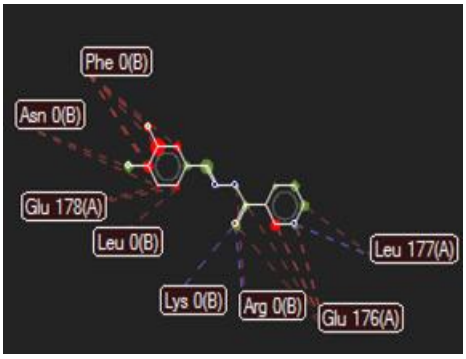
RK 5-Score:-8.04772 Kcal/Mol



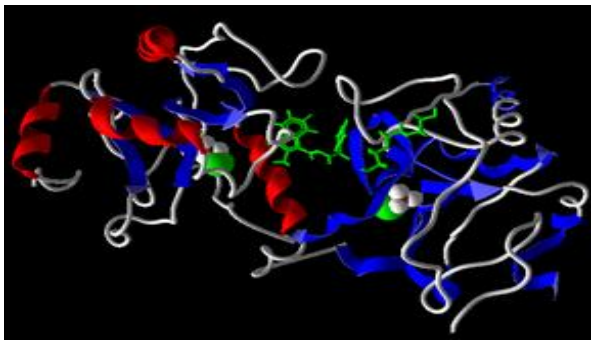


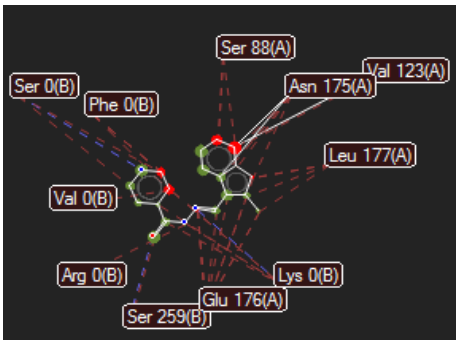
RK 6-Score:-9.86919 Kcal/Mol



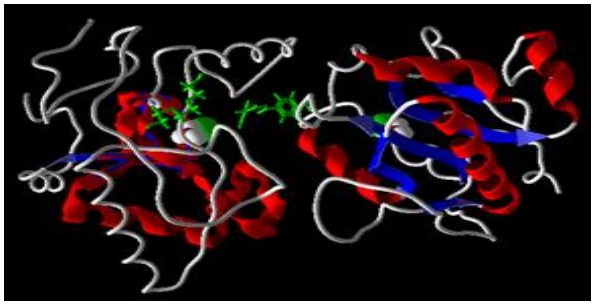


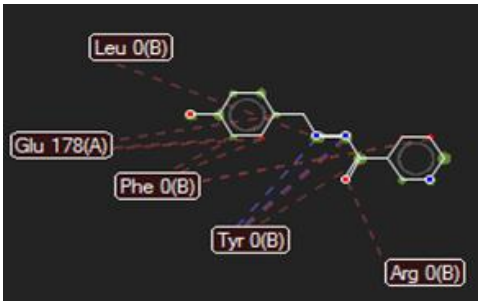
RK 7-Score:-8.81896 Kcal/Mol





RK 8-Score:-9.4802 Kcal/Mol





RK 9-Score:-7.15868 Kcal/Mol

RESULTS OF SYNTHETIC SCHEME

The compounds for the synthesis were chosen based on the high G-Score and their feasibility in synthetic chemistry. The series of compounds RK1, RK2, RK2a, RK3, RK4, RK5, RK6, RK7, RK8 and RK9 were synthesised and its physico chemical properties were observed.

RESULTS OF CHARACTERIZATION

The synthesised molecules were characterized by Infrared Spectroscopy, H^1 NMR Spectroscopy and GC-MS, LC-MS methods.

IR SPECTROSCOPY:

IR Spectra mainly used in structural elucidation to determine the various functional groups. Parent molecules (Primary amine and Aldehyde) shown a strectching vibration $-NH_2$ and C=O with their respective wave numbers 3500 – 3450 cm^{-1} and 1740 – 1720 cm^{-1}.

The series of synthesised products (RK1, RK2, RK2a, RK3, RK4, RK5, RK6, RK7, RK8, and RK9) showed a C=N strectching vibration (Ring out) with their respective wave number 2360.87 cm^{-1} with also indicates the absence of parent molecules functional groups with their respective wave numbers (cm^{-1}) which indicates the confirmation Schiff base formation.

GC–MS AND LC-MS SPECTROMETRY:

GC-MS Spectra is a hyphenated technique. Hence from the GC-MS Spectra given the following details of synthesised compounds.

- Determination of molecular mass

- Verifying the identity and purity of the known substance providing data on isotopic abundance.

LC-MS Spectra opted for characterization of the compound RK1. It were showed broad peak in the absorption spectra of GC-MS. The purity of the compound were same both in GC-MS and LC-MS.

NMR SPECTROSCOPY :

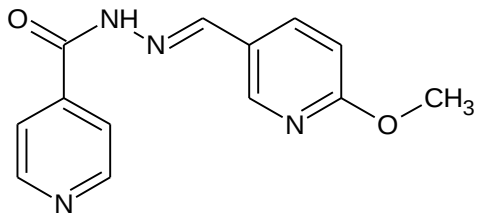
NMR Spectroscopy is especially used in structural elucidation of organic compound. Organic compounds invariably have hydrogen atoms in their structure and the environment of each proton is not same. Hence from the H^1 NMR spectra all the types of protons were observed from the structure. The H^1 NMR spectra given the details about,

- Types of protons
- No of each type of protons (in **δ** value)
  Aromatic proton: 6.5-9.85ppm
  Aliphatic proton: 2.3-3.85ppm
  Hydroxy proton: 4.3-7.7ppm
  Methyl proton: 6-7.9ppm
  Secondary amine proton: 2-5ppm (usually broad peak)
  CH=NH type proton: 11.8-13.2ppm
- Environment of protons (Position of the peak)
- No of adjacent protons
  (singlet,doublet,triplet,quartet,pentatet,sextet,multiplet)

The physiochemical properties profile and characterization results of synthesised copounds RK1, RK2, RK2a, RK3, RK4, RK5, RK6, RK7, RK8, and RK9 were observed.

REPORTS OF PRODUCT PROFILE AND CHARACTERIZATION

1. N'-[(E)-(6-methoxypyridin-3-yl)methylidene]pyridine-4-carbohydrazide

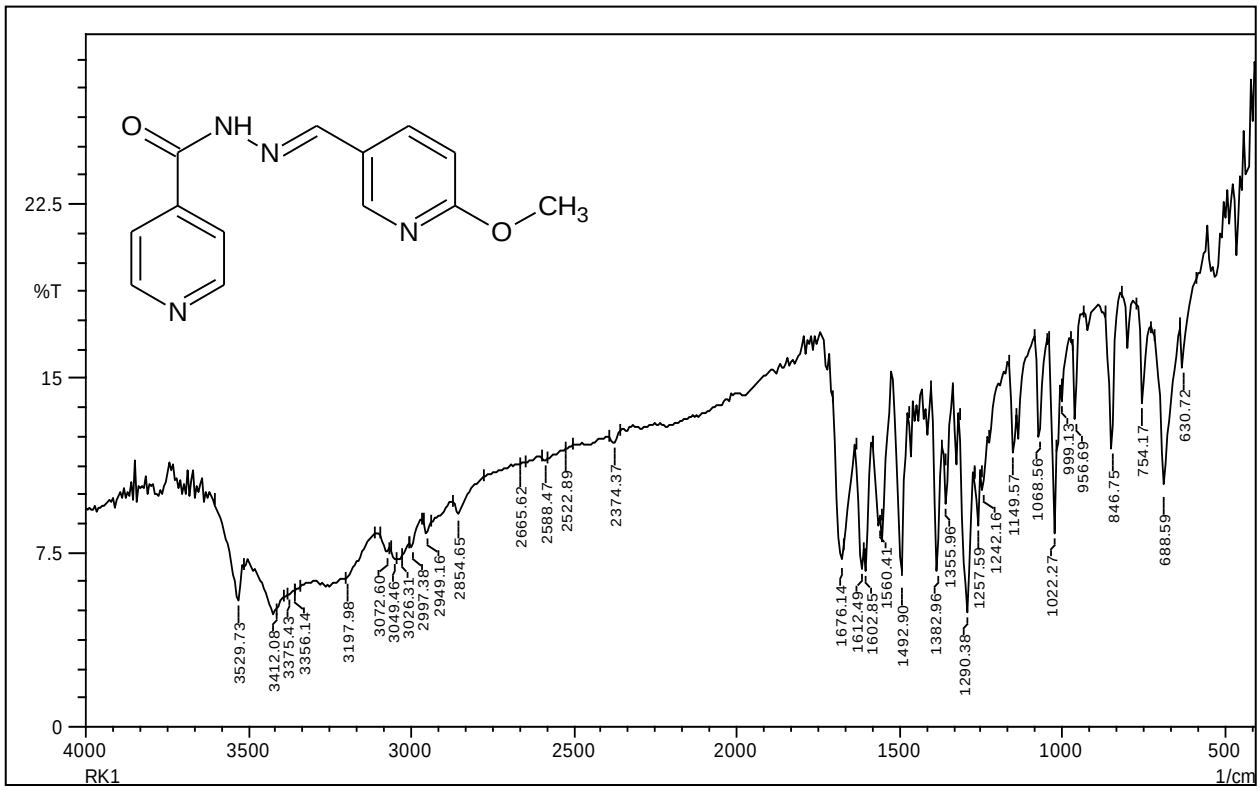


RK1

PHYSICO-CHEMICAL PROPERTIES

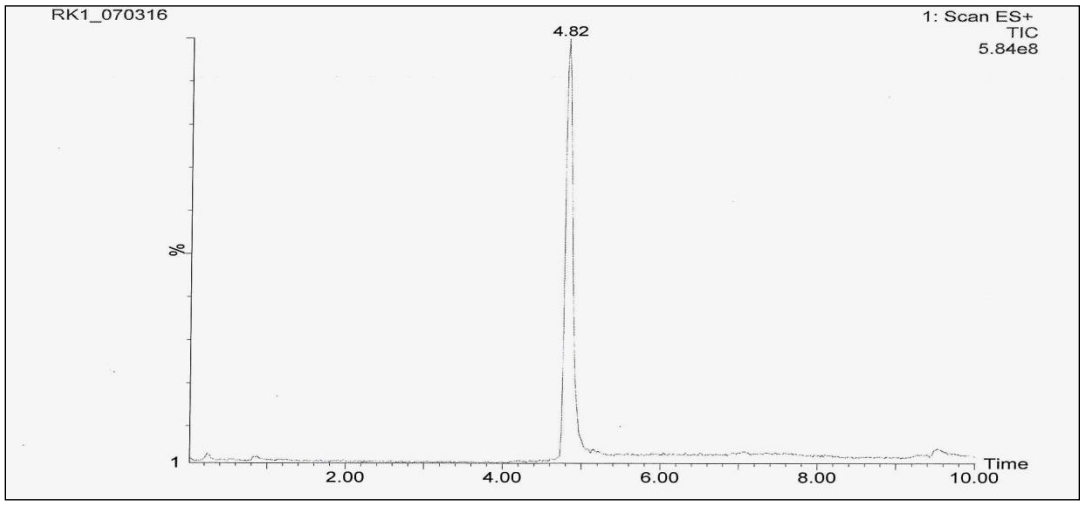
Description	:	Light Yellowish white Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	165°C
Molecular Formula	:	C13H12N4O2
Formula Weight	:	256.2599 g/Mol
Composition	:	C(60.93%) H(4.72%) N(21.86%) O(12.49%)
Molar Refractivity	:	71.63 ± 0.5 cm3
Molar Volume	:	206.6± 7.0 cm3
Parachor	:	543.0± 8.0 cm3
Index of Refraction	:	1.609± 0.05
Surface Tension	:	47.6 ± 7.0 dyne/cm
Density	:	1.24 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	28.39± 0.5 10-24cm3
Monoisotopic Mass	:	256.096026 Da
Monoisotopic Mass	:	256.096026 Da
Nominal Mass	:	256 Da
Average Mass	:	256.26 Da
M+	:	256.095477 Da
M-	:	256.096574 Da
[M+H]+	:	257.103302 Da
[M+H]-	:	257.104399 Da
[M-H]+	:	255.087652 Da
[M-H]-	:	255.088749 Da

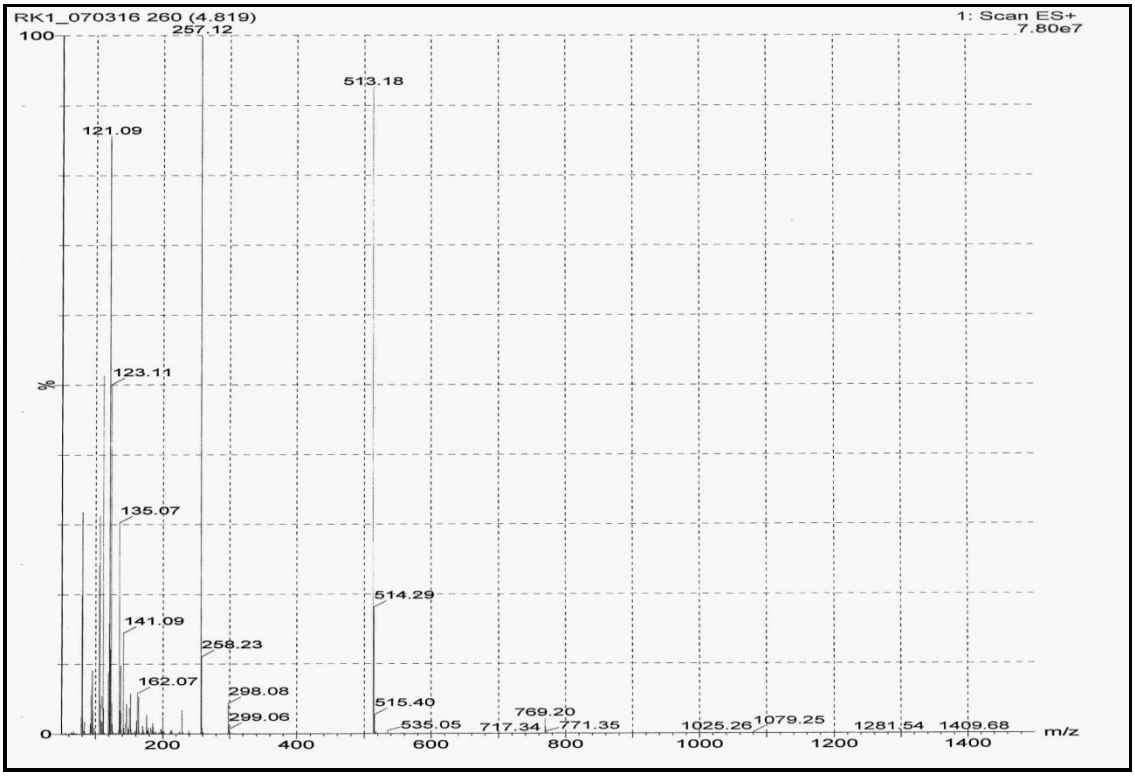
RK 1: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3049.46, 3072.6	C-H stretching	Aromatic
1676.14	C=O stretching	Amide
3412.08	N-H stretching	Amide
1602.95	C=N stretching	Aromatic (Ring in)
2374.37	C=N stretching	Aromatic (Ring out)
2854.65	C-O-C stretching	Methoxy group.
1492.90	C=C stretching	Aromatic

RK 1: LC-MS SPECTRUM

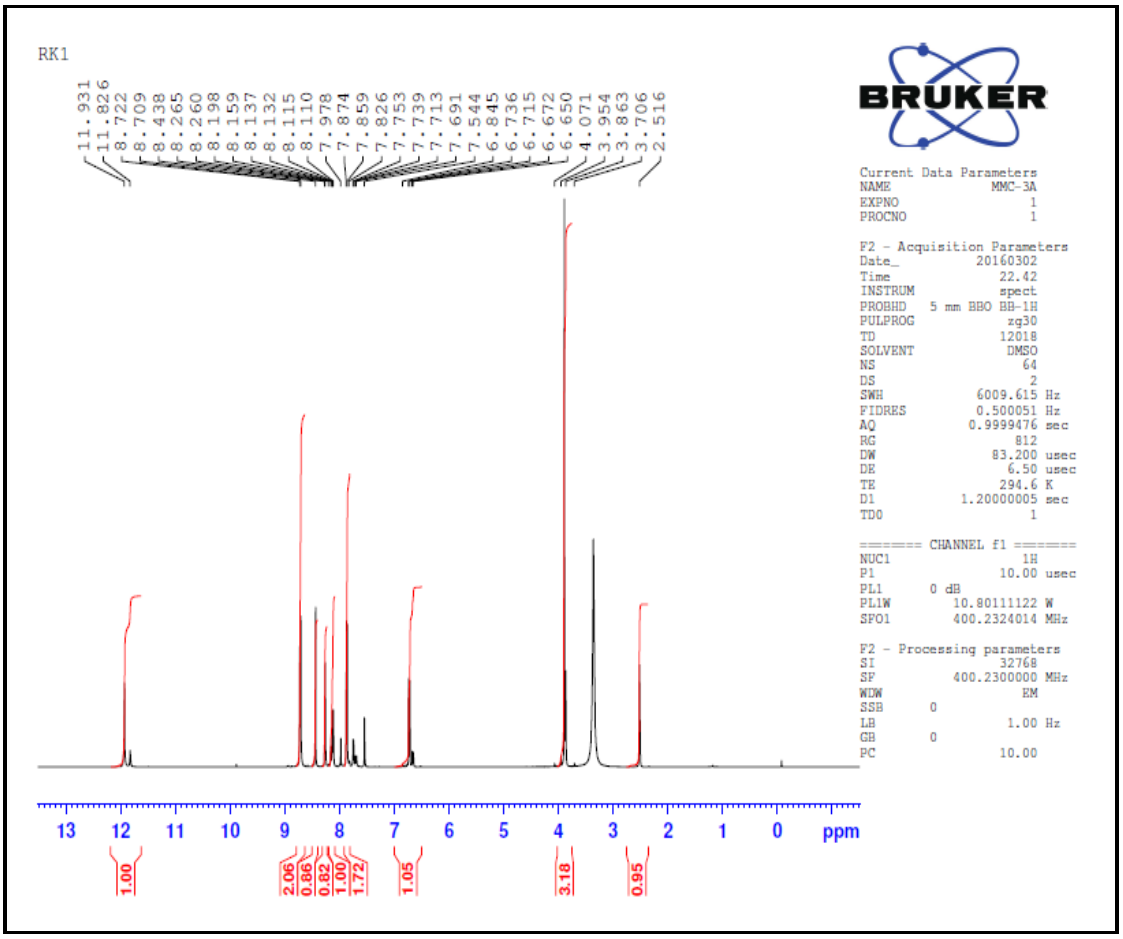




Actual Molecular Mass : 256.25 g/Mol

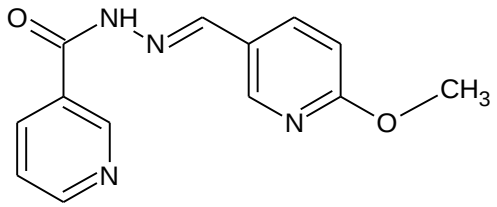
Expected Molecular Mass: 257.12 g/Mol

RK 1: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
1	Singlet	2.55ppm
3	Singlet	3.85ppm
1	Doublet	6.75-6.85ppm
6	Multiplet	7.55-8.55ppm
1	Doublet	11.9-12ppm

2. N'-[(E)-(6-methoxypyridin-3-yl)methylidene]pyridine-3- carbohydrazide

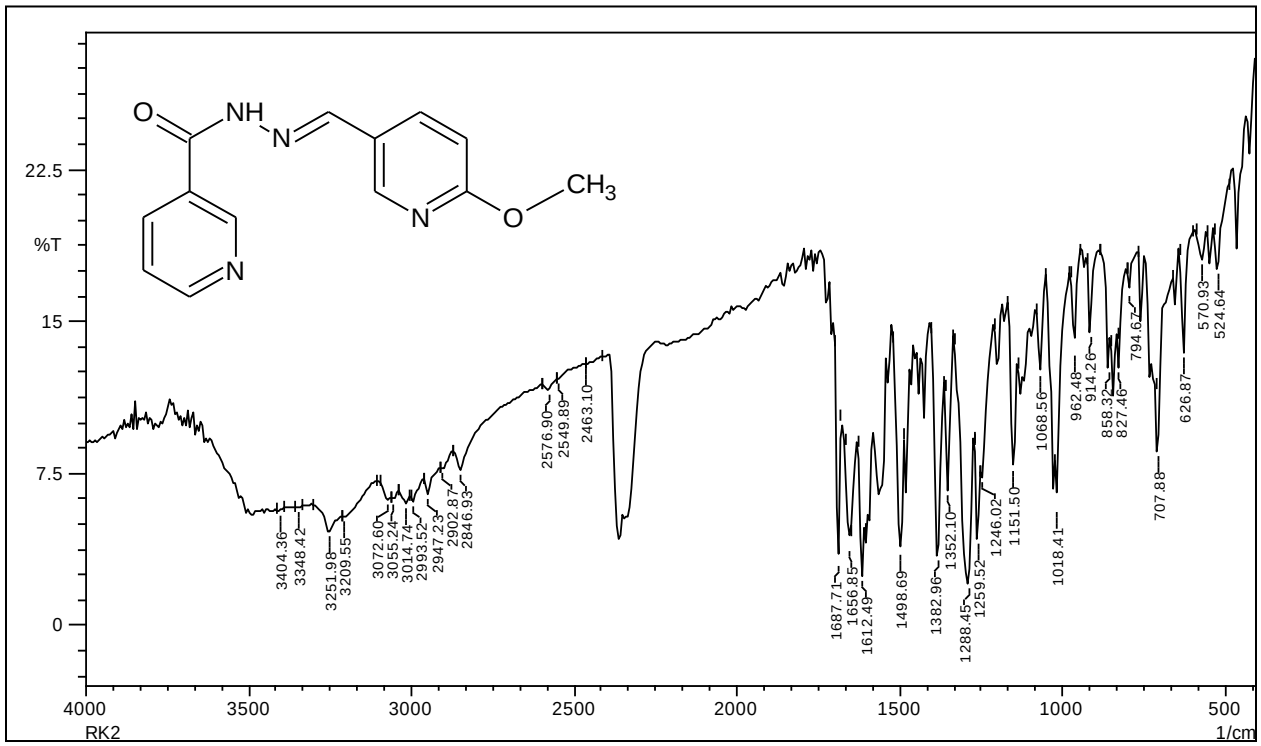


RK2

PHYSICO-CHEMICAL PROPERTIES

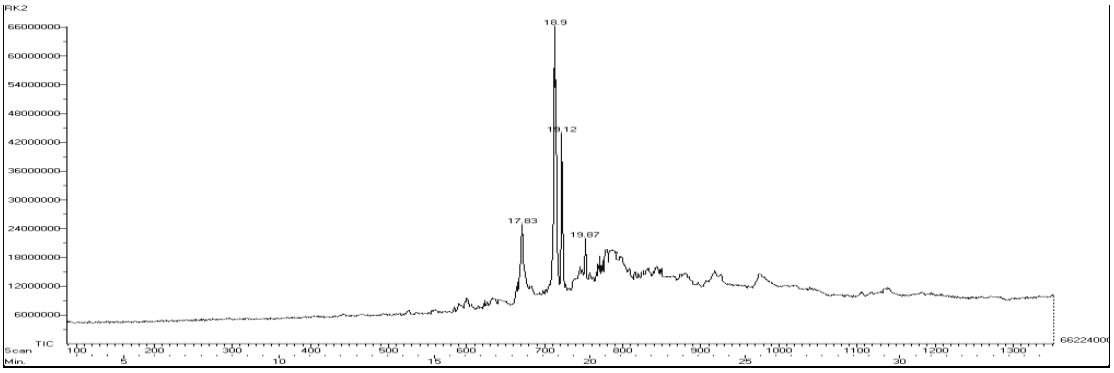
Description	:	Light Yellowish White Powder
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	193°C
Molecular Formula	:	C13H12N4O2
Formula Weight	:	256.25998 g/Mol
Composition	:	C(60.93%) H(4.72%) N(21.86%) O(12.49%)
Molar Refractivity	:	71.63 ± 0.5 cm3
Molar Volume	:	206.6± 7.0 cm3
Parachor	:	543.0± 8.0 cm3
Index of Refraction	:	1.609± 0.05
Surface Tension	:	47.6 ± 7.0 dyne/cm
Density	:	1.24 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	28.39± 0.5 10-24cm3
Monoisotopic Mass	:	256.096026 Da
Nominal Mass	:	256 Da
Average Mass	:	256.26 Da
M+	:	256.095477 Da
M-	:	256.096574 Da
[M+H]+	:	257.103302 Da
[M+H]-	:	257.104399 Da
[M-H]+	:	255.087652 Da
[M-H]-	:	255.088749 Da

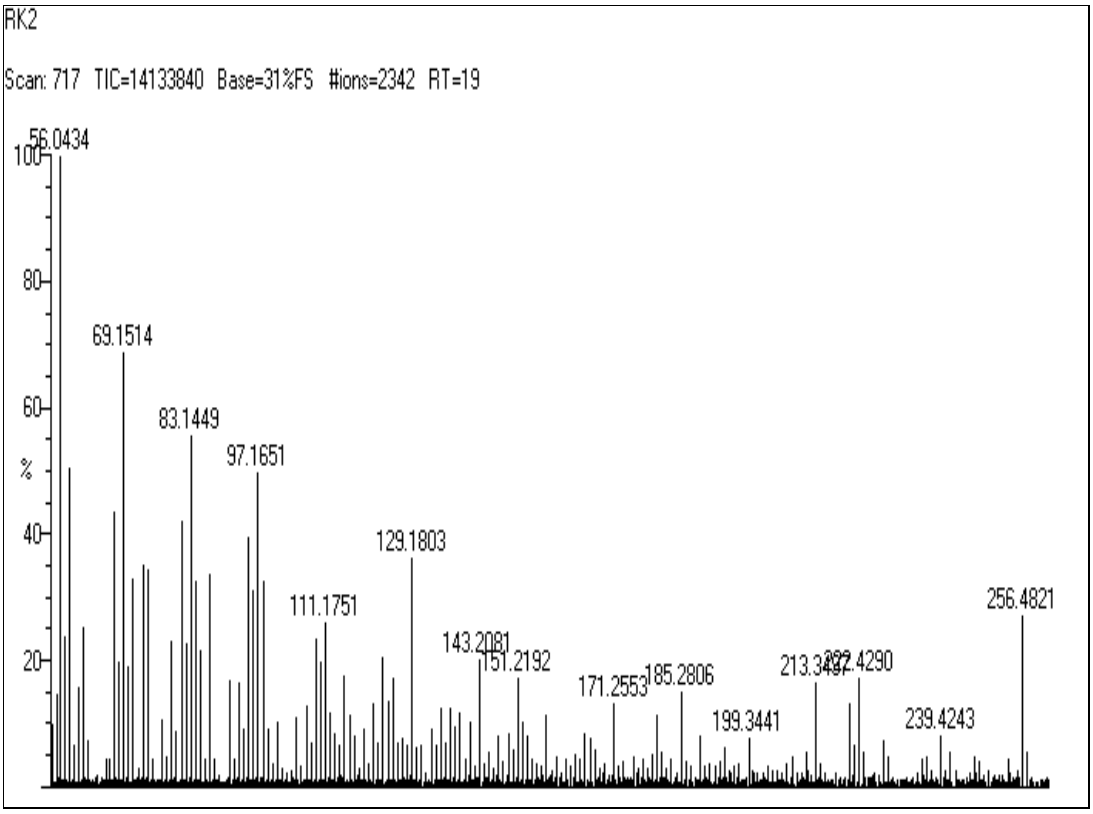
RK 2: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3072.60, 3014.74	C-H stretching	Aromatic
1687.71	C=O stretching	Amide
3404.36	N-H stretching	Amide
1612.49	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
2846.93	C-O-C stretching	Methoxy group.
1498.69	C=C stretching	Aromatic

RK 2: GC-MS SPECTRUM

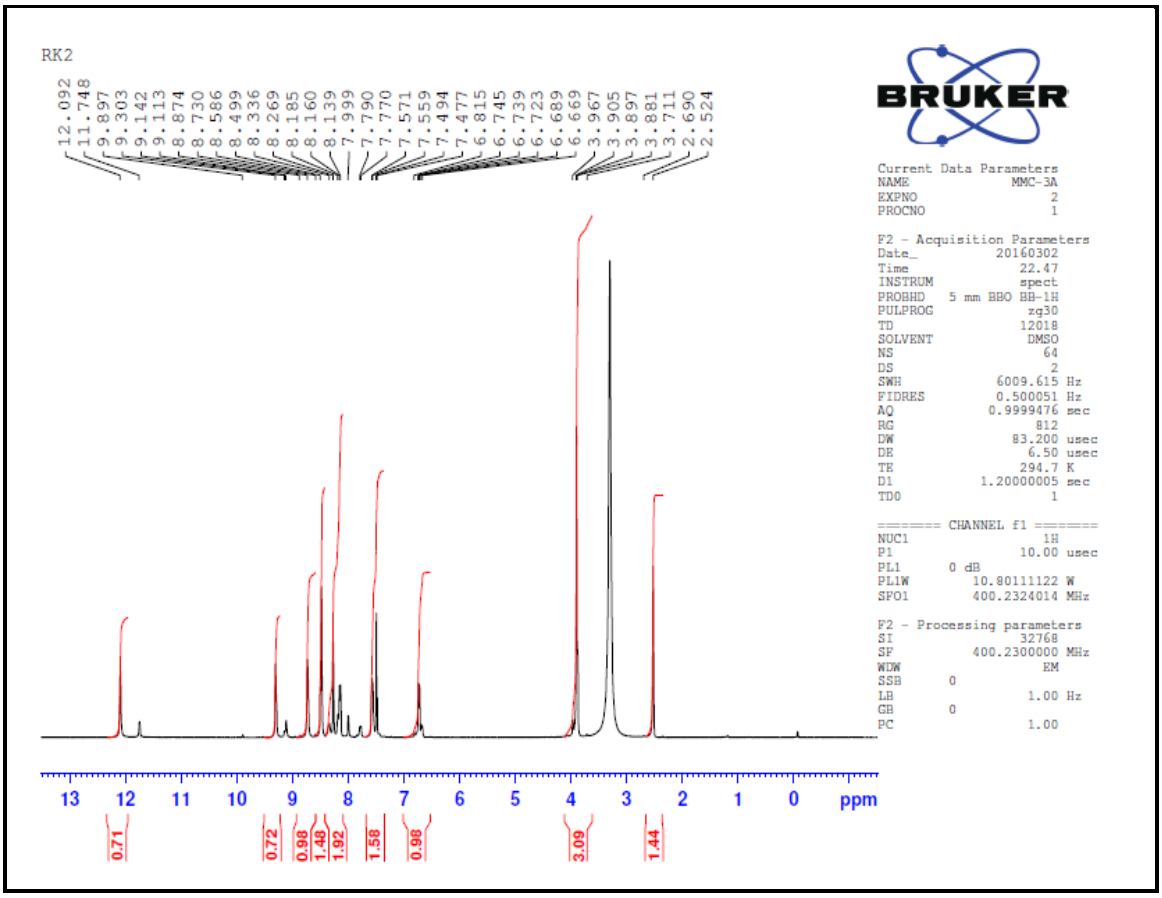




Actual Molecular Mass : 256.25 g/Mol

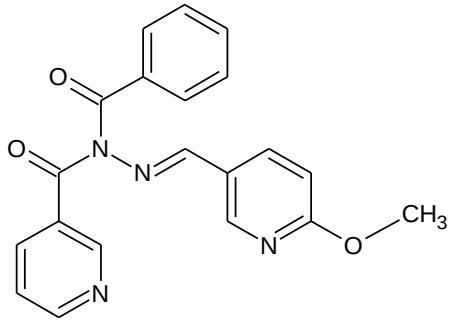
Expected Molecular Mass: 256.48 g/Mol

RK 2: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
1	Singlet	2.55ppm
3	Singlet	3.85ppm
1	Doublet	6.75-6.85ppm
6	Multiplet	7.5-9.3ppm
1	Singlet	12.2ppm

3. N-benzoyl-N'-[(E)-(6-methoxypyridin-3- yl)methylidene]pyridine-3- Carbohydrazide

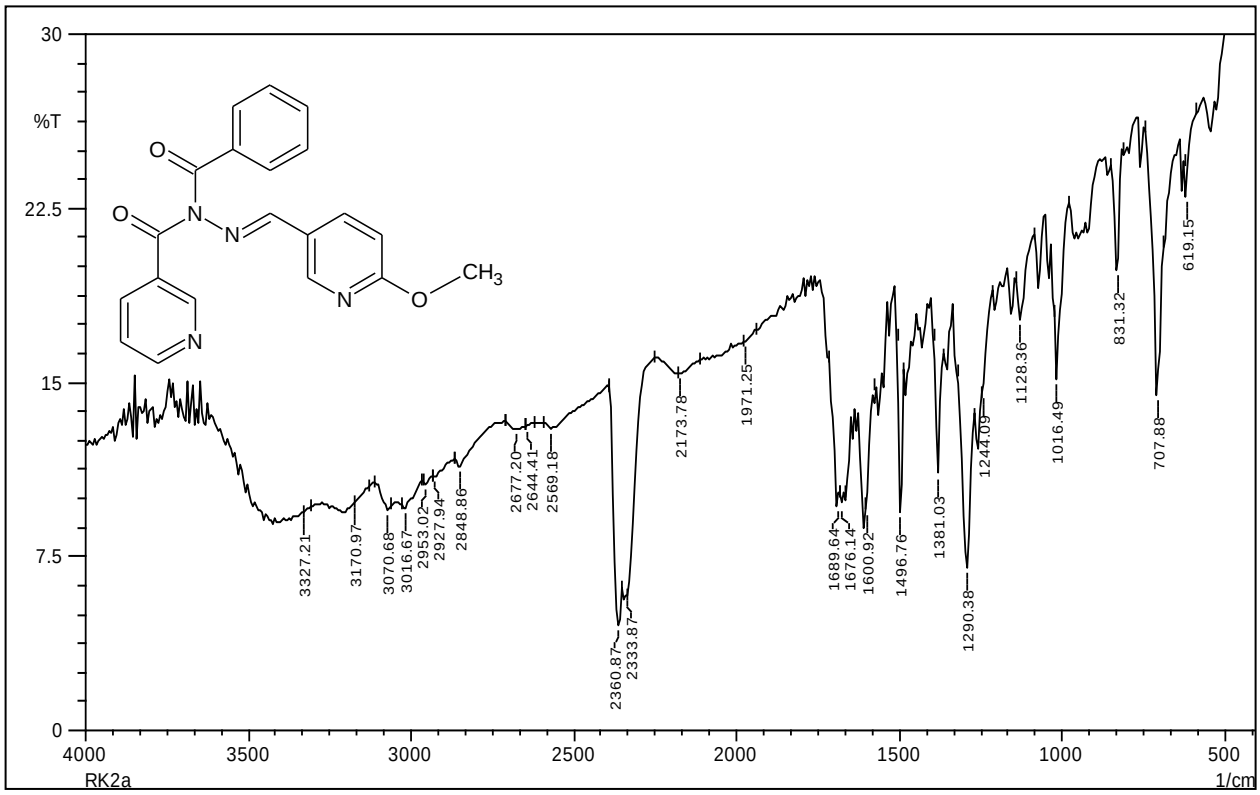


RK2a

PHYSICO-CHEMICAL PROPERTIES

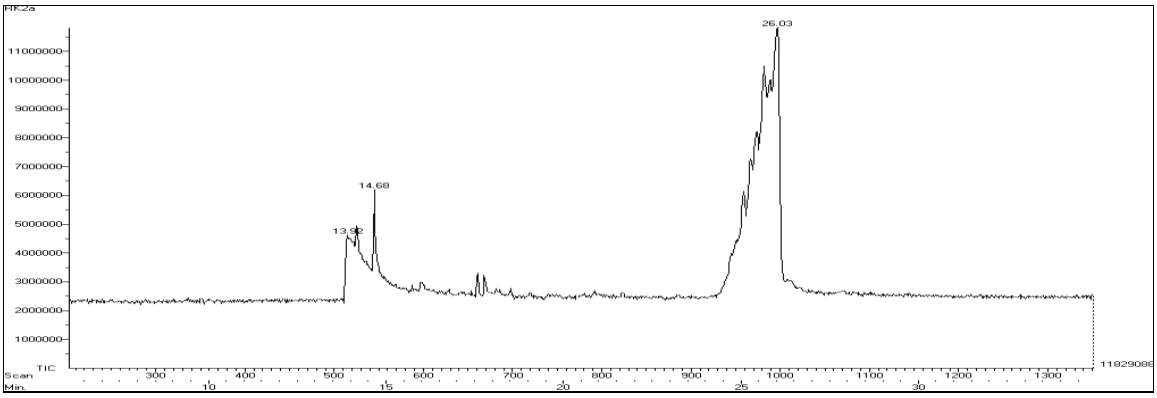
Description	:	Pale Yellow Gleaming solid
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	88°C
Molecular Formula	:	C20H16N4O3
Formula Weight	:	360.36604 g/Mol
Composition	:	C(66.66%) H(4.48%) N(15.55%) O(13.32%)
Molar Refractivity	:	103.09 ± 0.5 cm3
Molar Volume	:	294.0± 7.0 cm3
Parachor	:	778.5± 8.0 cm3
Index of Refraction	:	1.618± 0.05
Surface Tension	:	49.1 ± 7.0 dyne/cm
Density	:	49.1 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	49.1± 0.5 10-24cm3
Monoisotopic Mass	:	360.12224 Da
Nominal Mass	:	360 Da
Average Mass	:	360.366 Da
M+	:	360.121692 Da
M-	:	360.122789 Da
[M+H]+	:	361.129517 Da
[M+H]-	:	361.130614 Da
[M-H]+	:	359.113867 Da
[M-H]-	:	359.114964 Da

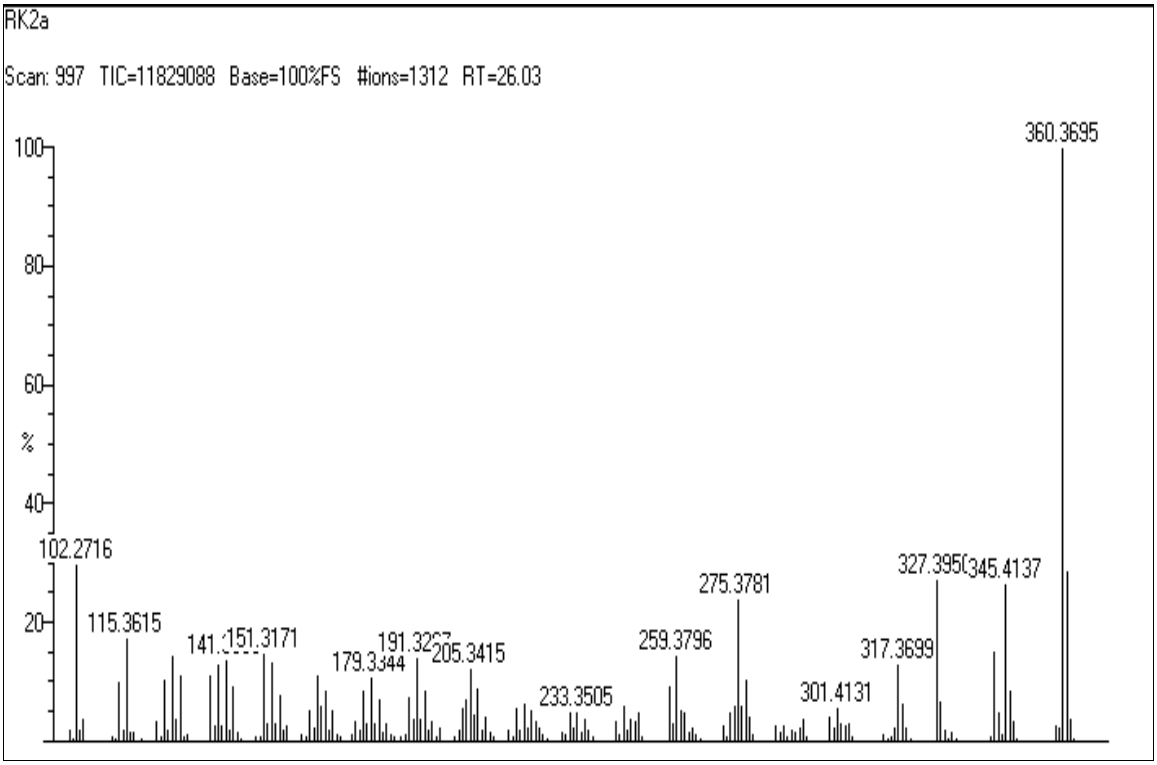
RK 2a: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3070.97, 3016.67	C-H stretching	Aromatic
1689.64	C=O stretching	Amide
3327.21	N-H stretching	Amide
1600.92	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
2848.86	C-O-C stretching	Methoxy group.
1496.76	C=C stretching	Aromatic

RK 2a: GC-MS SPECTRUM

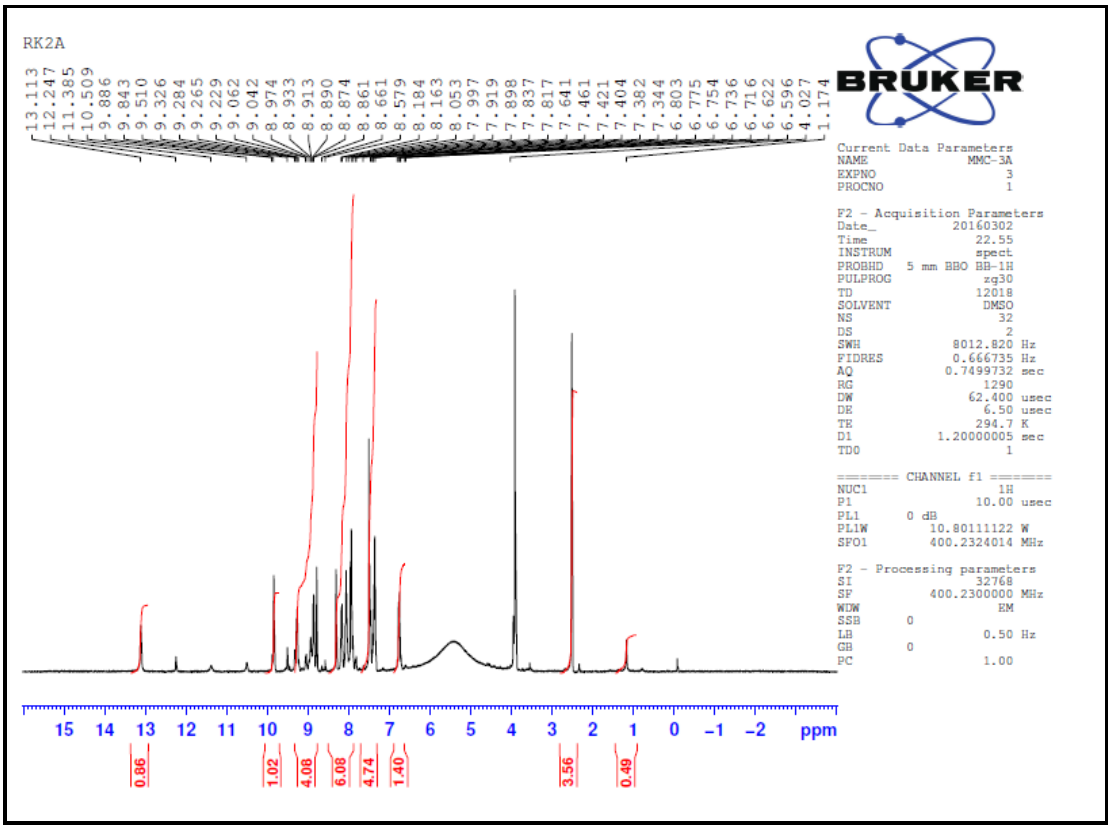




Actual Molecular Mass : 360.36 g/Mol

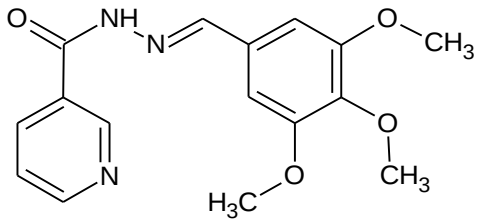
Expected Molecular Mass: 360.36 g/Mol

RK 2a: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
3	Doublet	2.3-2.5ppm
1	Doublet	6.7-6.85ppm
4	Doublet	7.35-7.5ppm
6	Sextet	7.95-8.3ppm
5	Multiplet	8.7-9.9ppm
1	Singlet	13.2ppm

4. N'-[(E)-(3,4,5-trimethoxyphenyl)methylidene]pyridine-3-carbohydrazide

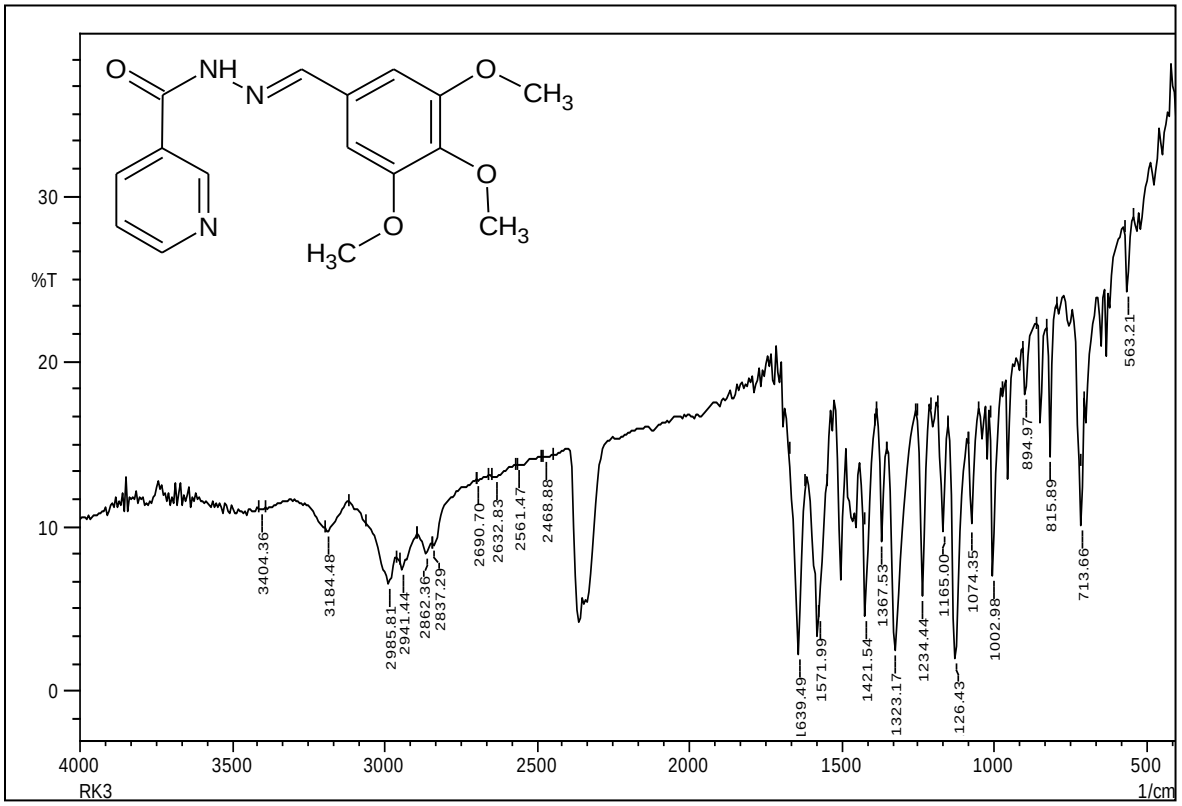


RK3

PHYSICO-CHEMICAL PROPERTIES

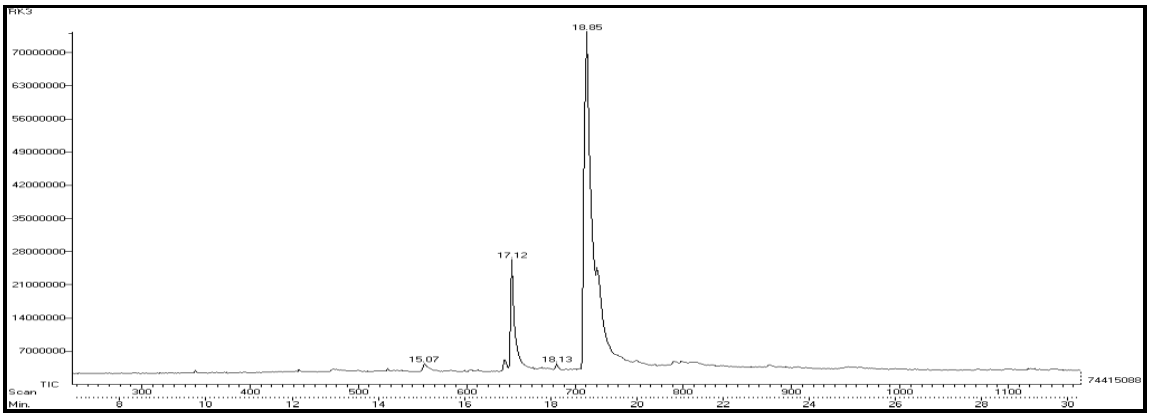
Description	:	Milky White Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	178°C
Molecular Formula	:	C16H17N3O4
Formula Weight	:	315.32388 g/Mol
Composition	:	C(60.94%) H(5.43%) N(13.33%) O(20.30%)
Molar Refractivity	:	84.81 ± 0.5 cm3
Molar Volume	:	261.4± 7.0 cm3
Parachor	:	662.4± 8.0 cm3
Index of Refraction	:	1.562± 0.05
Surface Tension	:	41.2 ± 7.0 dyne/cm
Density	:	1.20 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	33.62± 0.5 10-24cm3
Monoisotopic Mass	:	315.121906 Da
Nominal Mass	:	315 Da
Average Mass	:	315.3239 Da
M+	:	315.121357 Da
M-	:	315.122455 Da
[M+H]+	:	316.129182 Da
[M+H]-	:	316.13028 Da
[M-H]+	:	314.113532 867 Da
[M-H]-	:	314.11463 Da

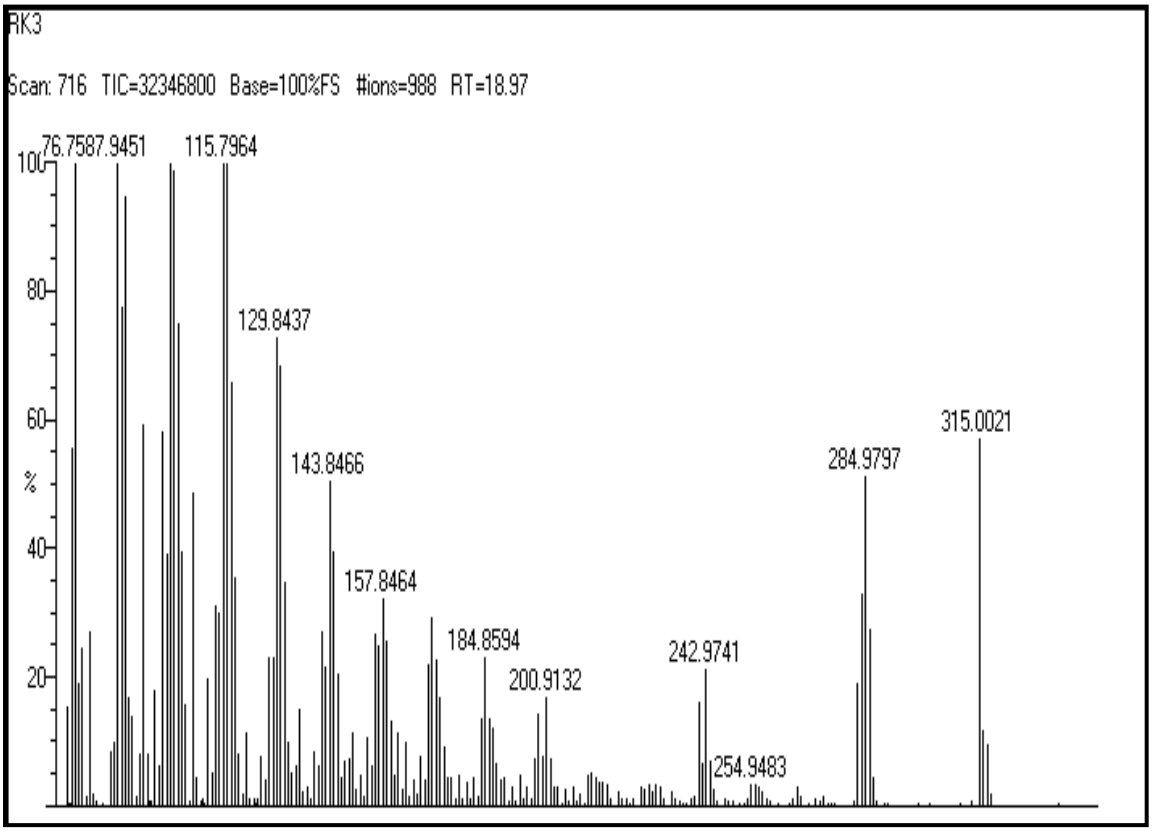
RK 3: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3184.48	C-H stretching	Aromatic
1639.48	C=O stretching	Amide
3404.36	N-H stretching	Amide
1571.99	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
2862.36	C-O-C stretching	Methoxy group.
1421.54	C=C stretching	Aromatic

RK 3: GC – MS SPECTRUM

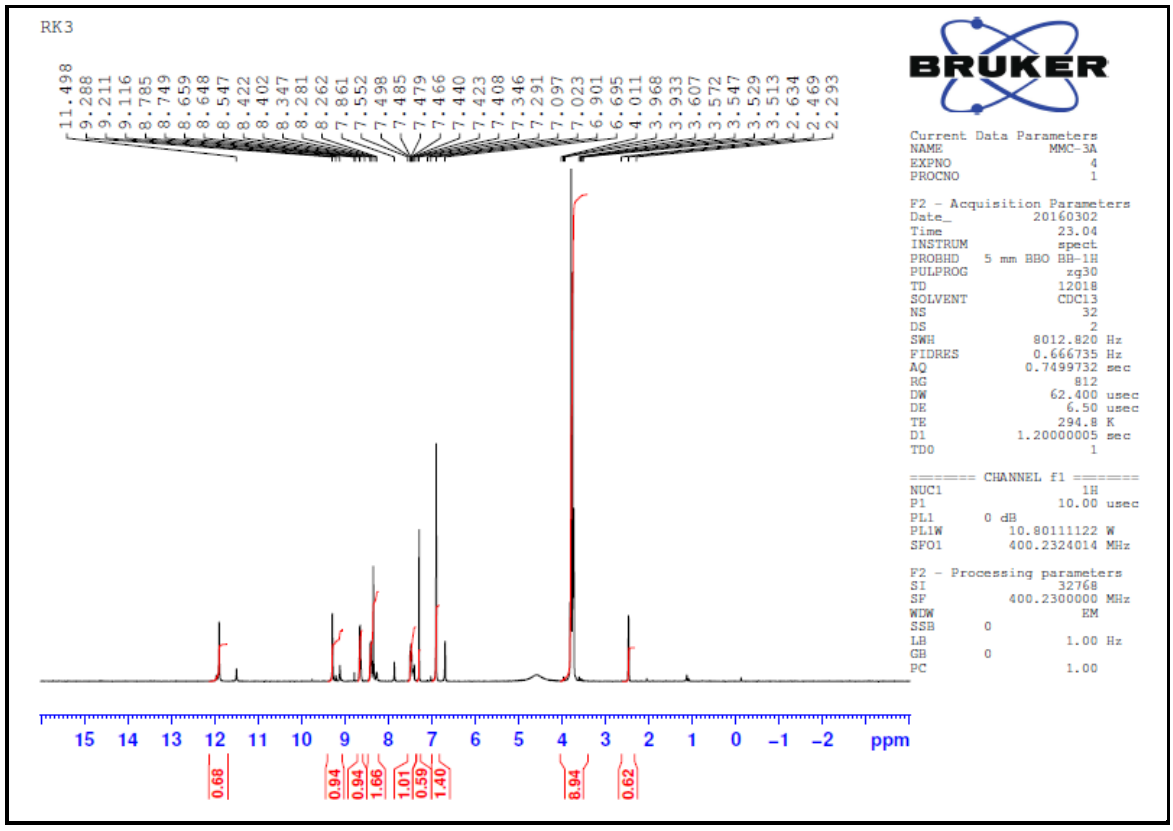




Actual Molecular Mass : 315.32 g/Mol

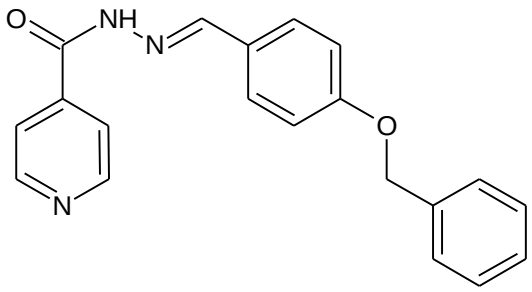
Expected Molecular Mass: 315.02 g/Mol

RK 3: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
1	Singlet	2.6ppm
9	Singlet	3.75-3.9ppm
6	Multiplet	6.9-9.3ppm
1	Singlet	11.9ppm

5. N'-{(E)-[4-(benzyloxy)phenyl]methylidene}pyridine-4-carbohydrazide

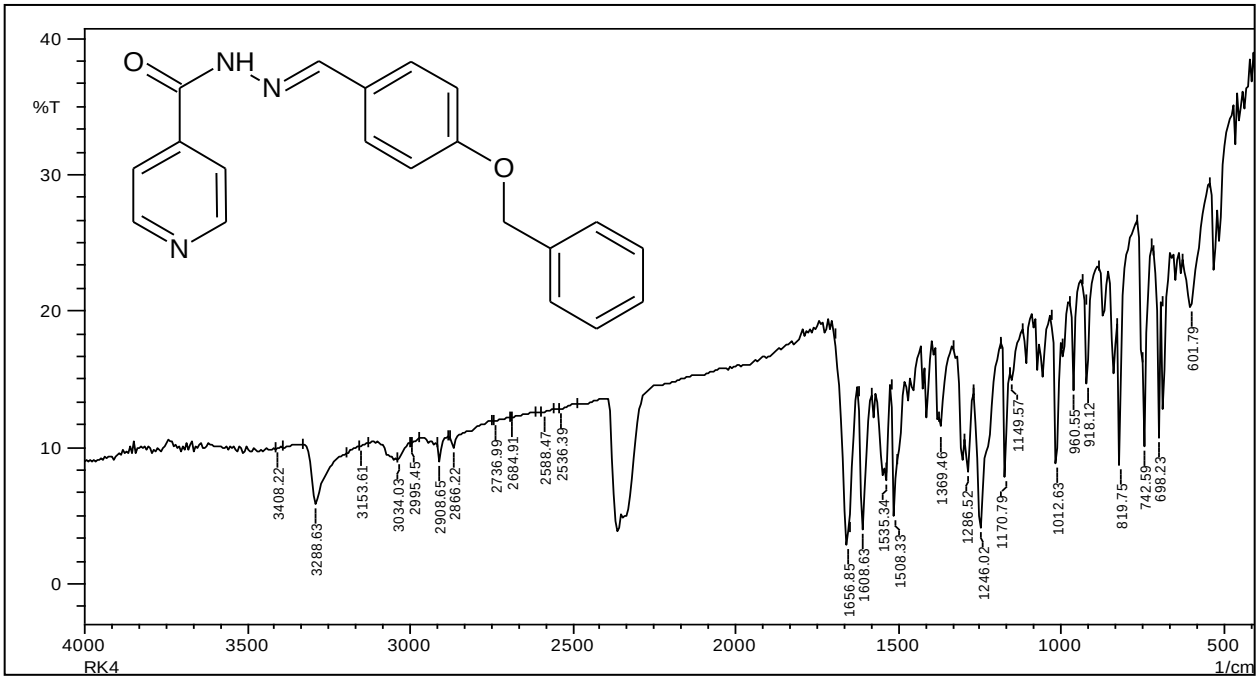


RK4

PHYSICO-CHEMICAL PROPERTIES

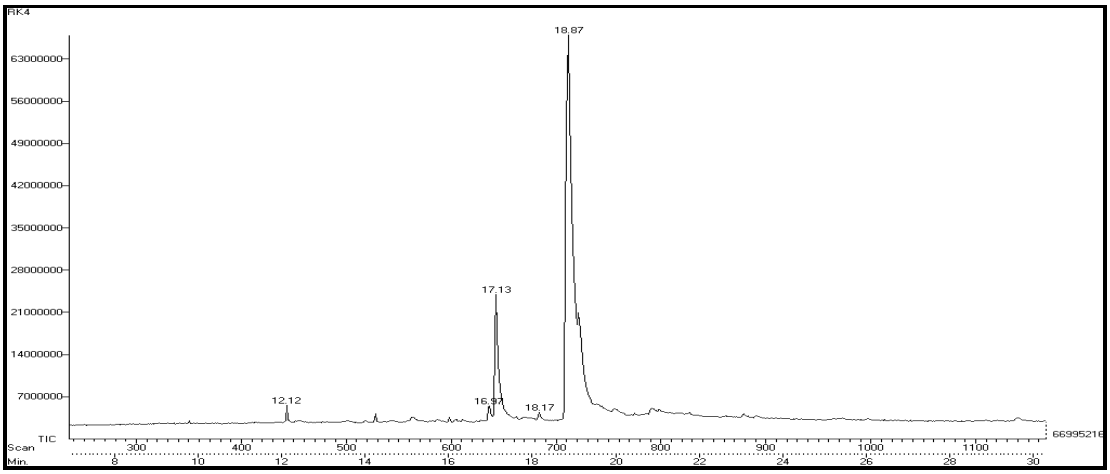
Description	:	Pale Yellowish Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	149°C
Molecular Formula	:	C20H17N3O2
Formula Weight	:	331.36788 g/Mol
Composition	:	C(72.49%) H(5.17%) N(12.68%) O(9.66%)
Molar Refractivity	:	98.47± 0.5 cm3
Molar Volume	:	286.8± 7.0 cm3
Parachor	:	746.2± 8.0 cm3
Index of Refraction	:	1.602± 0.05
Surface Tension	:	45.8 ± 7.0 dyne/cm
Density	:	1.15 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	39.03± 0.5 10-24cm3
Monoisotopic Mass	:	331.132077 Da
Nominal Mass	:	331 Da
Average Mass	:	331.3679 Da
M+	:	331.131528 Da
M-	:	331.132625 Da
[M+H]+	:	332.139353 Da
[M+H]-	:	332.14045 Da
[M-H]+	:	330.123703 Da
[M-H]-	:	330.1248 Da

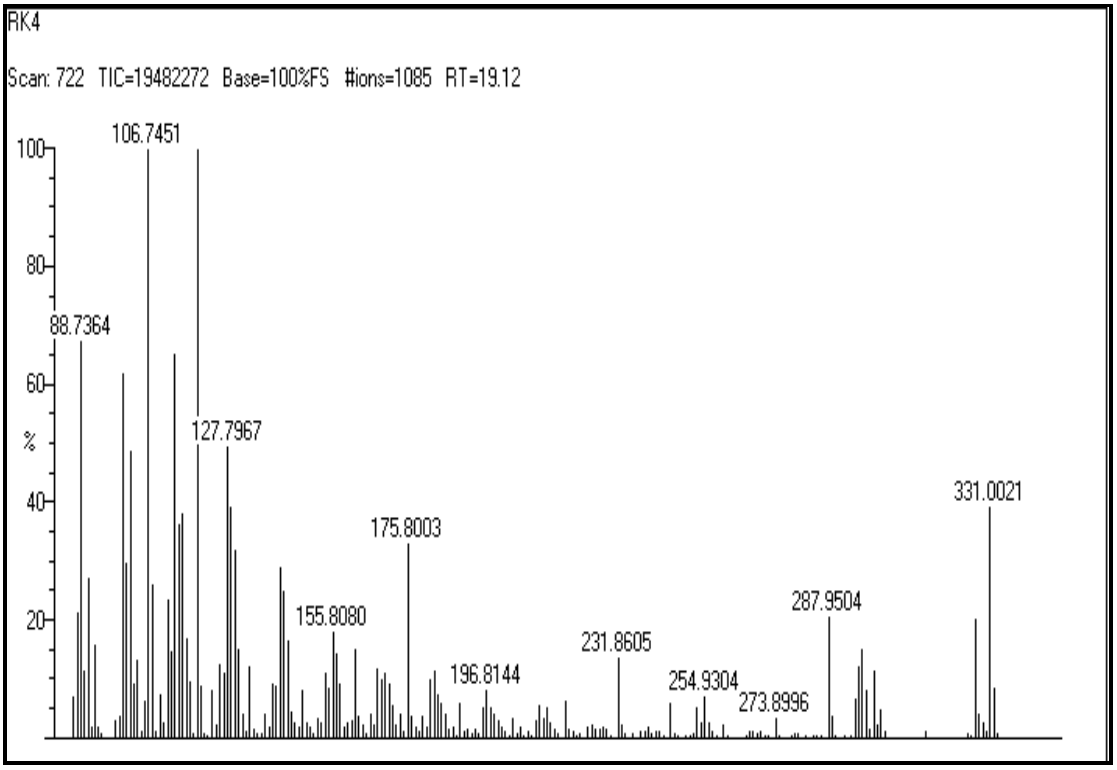
RK 4: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3034.03	C-H stretching	Aromatic
1656.85	C=O stretching	Amide
3408.22	N-H stretching	Amide
1608.63	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
2908.65	C-CH2 stretching	Methylene group.
1492.90	C=C stretching	Aromatic

RK 4: GC – MS SPECTRUM

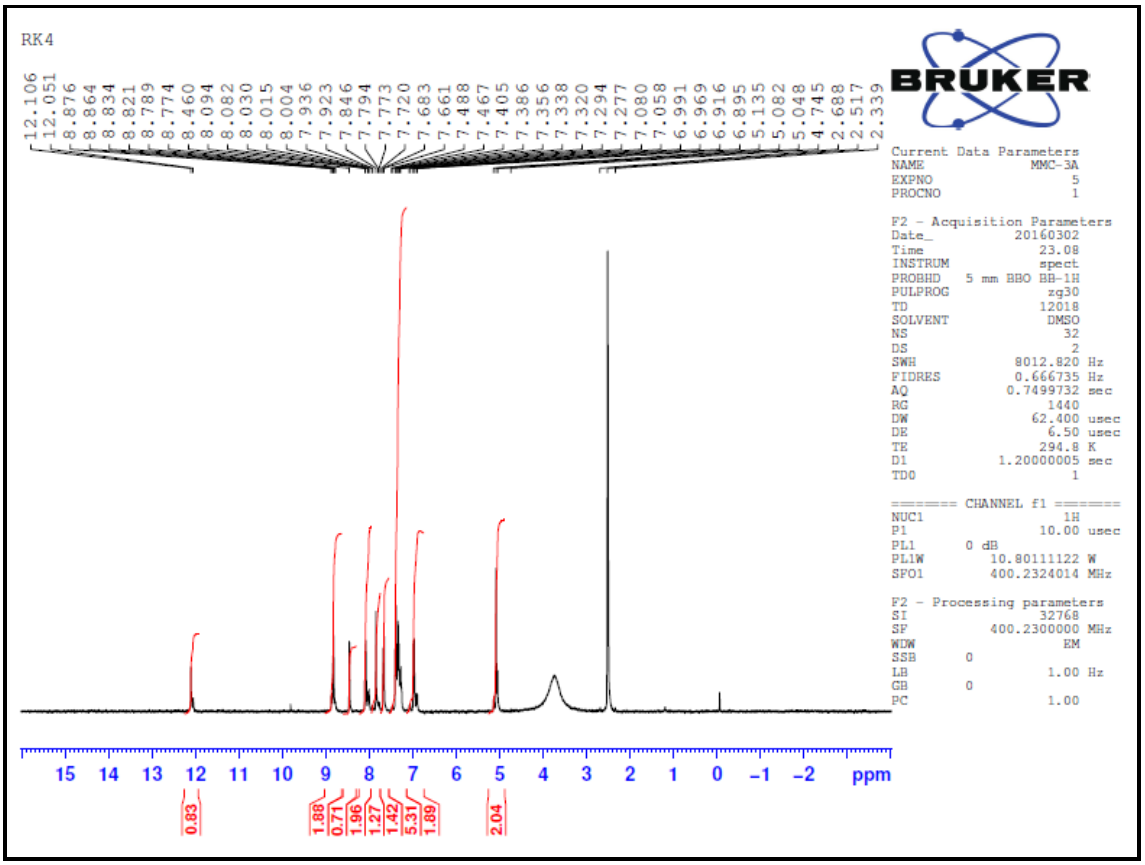




Actual Molecular Mass : 331.36 g/Mol

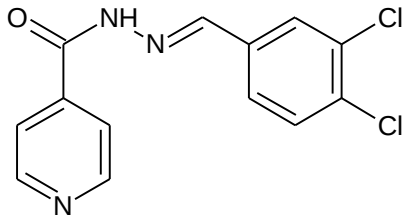
Expected Molecular Mass: 331.002 g/Mol

RK 4: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
2	Singlet	5.1ppm
14	Multiplet	6.9-8.9ppm
1	Singlet	12.1ppm

6. N-benzoyl-N'-[(1E,2E)-3-phenylprop-2-en-1-ylidene]pyridine-4- carbohydrazide

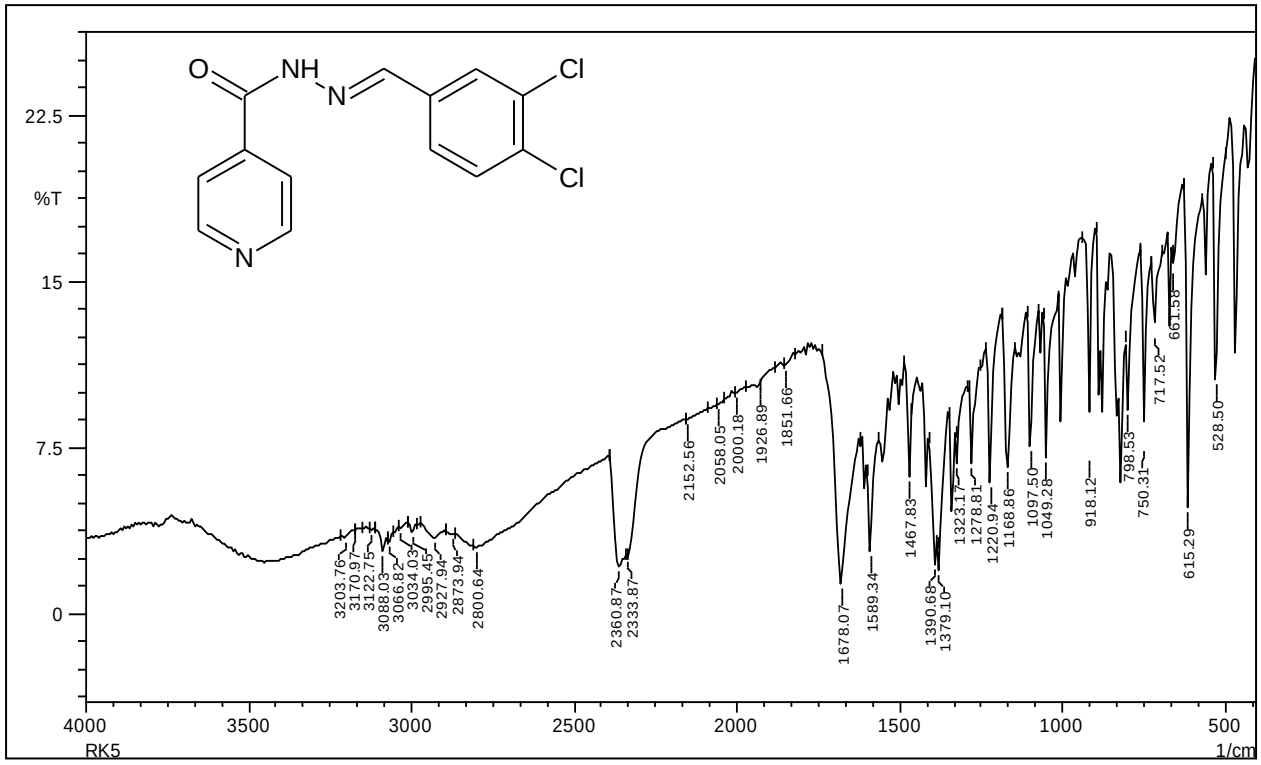


RK5

PHYSICO-CHEMICAL PROPERTIES

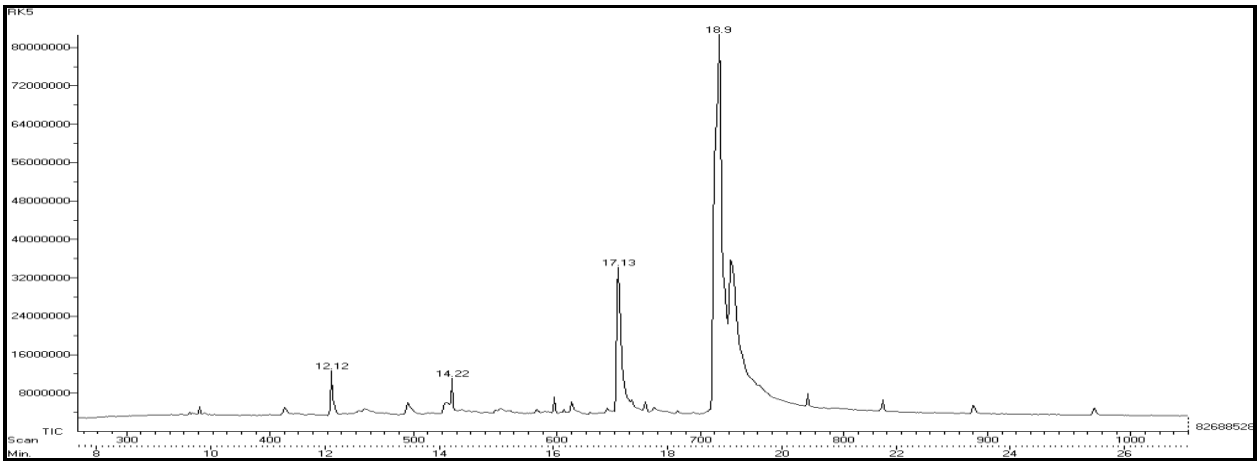
Description	:	Off Yellowish White Solid
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	189°C
Molecular Formula	:	C13H9Cl2N3O
Formula Weight	:	294.13606 g/Mol
Composition	:	C(53.08%) H(3.08%) Cl(24.11%) N(14.29%) O(5.44%)
Molar Refractivity	:	76.57 ± 0.5 cm3
Molar Volume	:	214.9± 7.0 cm3
Parachor	:	569.3± 8.0 cm3
Index of Refraction	:	1.631± 0.05
Surface Tension	:	49.2 ± 7.0 dyne/cm
Density	:	1.36 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	30.35± 0.5 10-24cm3
Monoisotopic Mass	:	293.012267 Da
Nominal Mass	:	293 Da
Average Mass	:	294.1361 Da
M+	:	293.011719 Da
M-	:	293.012816 Da
[M+H]+	:	294.019544 Da
[M+H]-	:	294.020641 Da
[M-H]+	:	292.003894 Da
[M-H]-	:	292.004991 Da

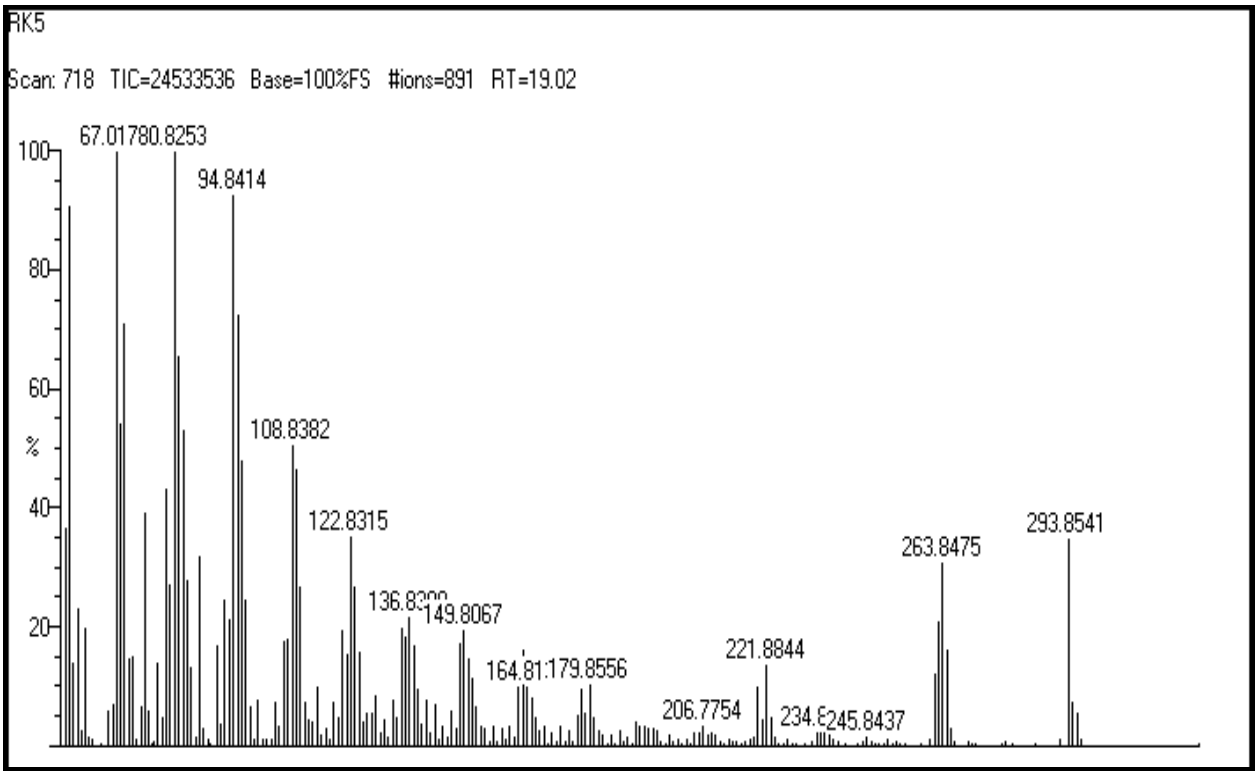
RK 5: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3088.03, 3066.82	C-H stretching	Aromatic
1678.07	C=O stretching	Amide
3203.76	N-H stretching	Amide
1589.34	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
750.31	C-Cl stretching	Halide group.
1390.68	C=C stretching	Aromatic

RK 5: GC – MS SPECTRUM

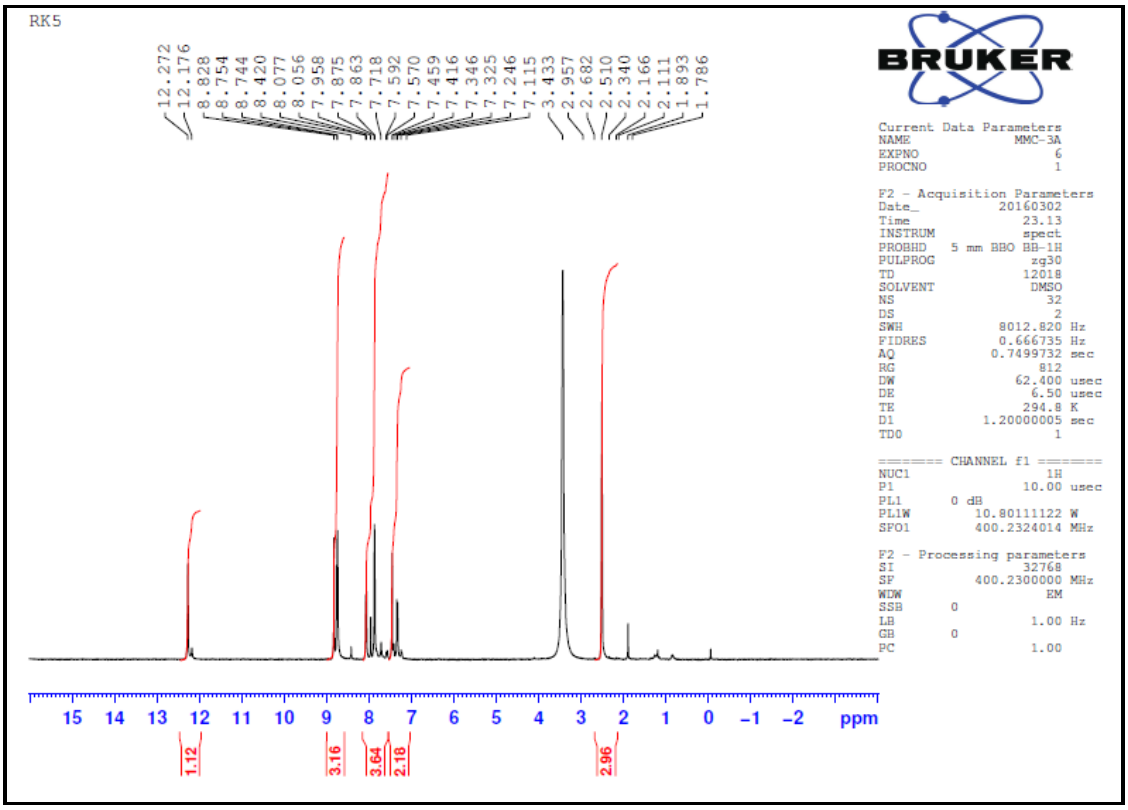




Actual Molecular Mass : 294.13 g/Mol

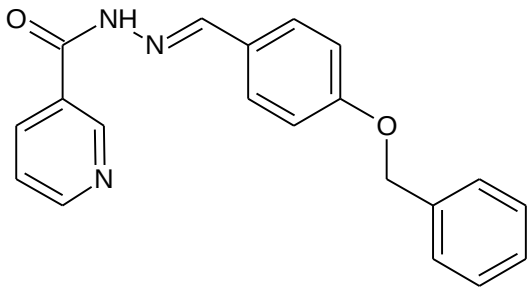
Expected Molecular Mass : 293.85 g/Mol

RK 5: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
2	Singlet	2.5ppm
5	Multiplet	7.2-8.1ppm
3	Doublet	8.7-8.9ppm
1	Doublet	12.3ppm

7. N'-{(E)-[4-(benzyloxy)phenyl]methylidene}pyridine-3-carbohydrazide

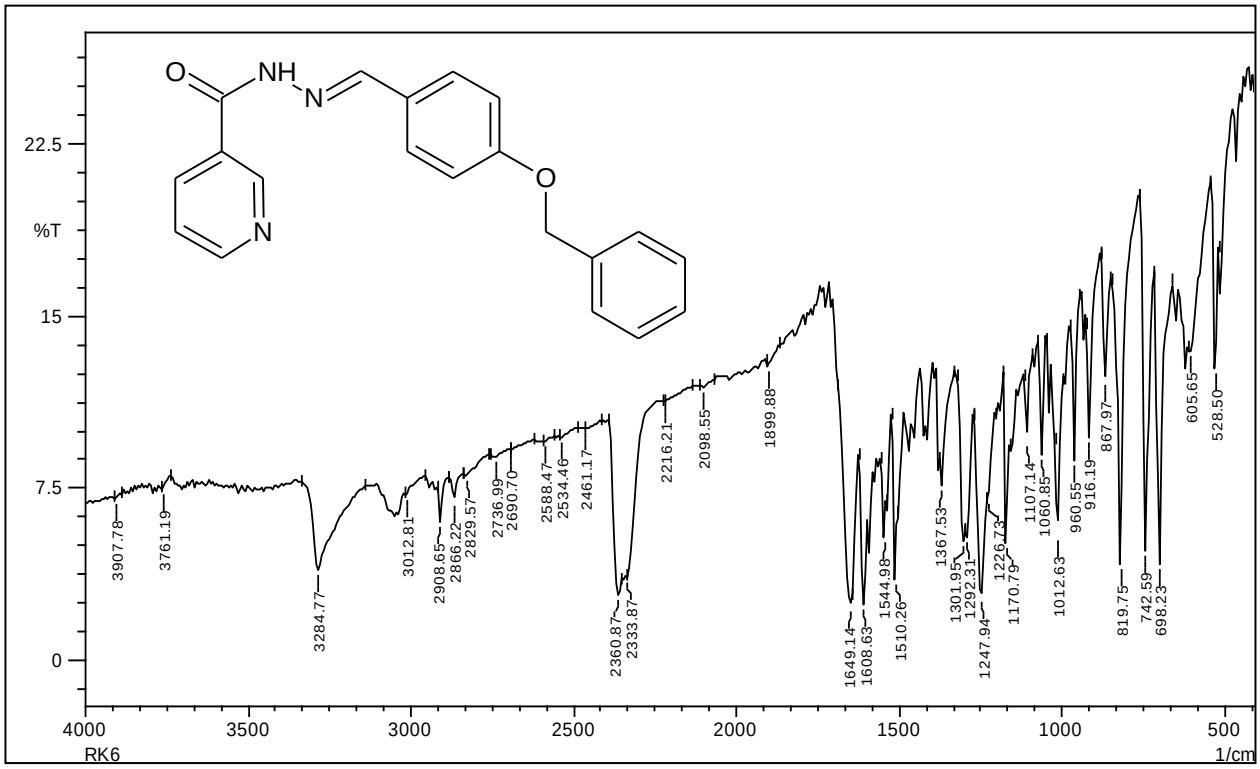


RK6

PHYSICO-CHEMICAL PROPERTIES

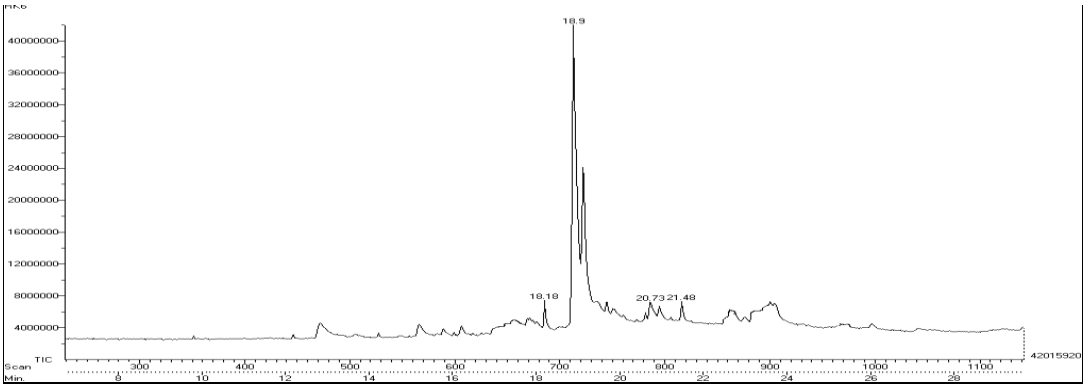
Description	:	Pale Yellowish Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	119°C
Molecular Formula	:	C20H17N3O2
Formula Weight	:	331.36788 g/Mol
Composition	:	C(72.49%) H(5.17%) N(12.68%) O(9.66%)
Molar Refractivity	:	98.47± 0.5 cm3
Molar Volume	:	286.8± 7.0 cm3
Parachor	:	746.2± 8.0 cm3
Index of Refraction	:	1.602± 0.05
Surface Tension	:	45.8 ± 7.0 dyne/cm
Density	:	1.15 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	39.03± 0.5 10-24cm3
Monoisotopic Mass	:	331.132077 Da
Nominal Mass	:	331 Da
Average Mass	:	331.3679 Da
M+	:	331.131528 Da
M-	:	331.132625 Da
[M+H]+	:	332.139353 Da
[M+H]-	:	332.14045 Da
[M-H]+	:	330.123703 Da
[M-H]-	:	330.1248 Da

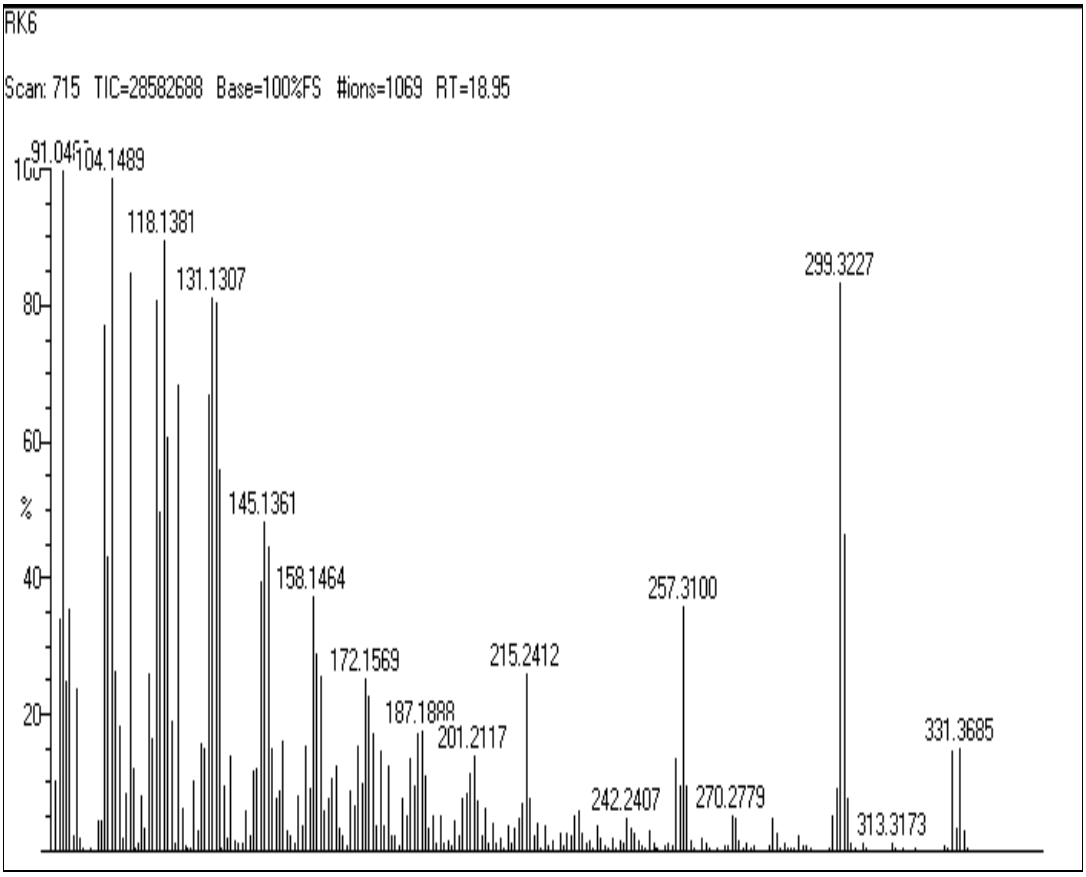
RK 6: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3012.81	C-H stretching	Aromatic
1649.14	C=O stretching	Amide
3284.77	N-H stretching	Amide
1608.63	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
2908.65	C-CH2 stretching	Methylene group.
1510.26	C=C stretching	Aromatic

RK 6: GC-MS SPECTRUM

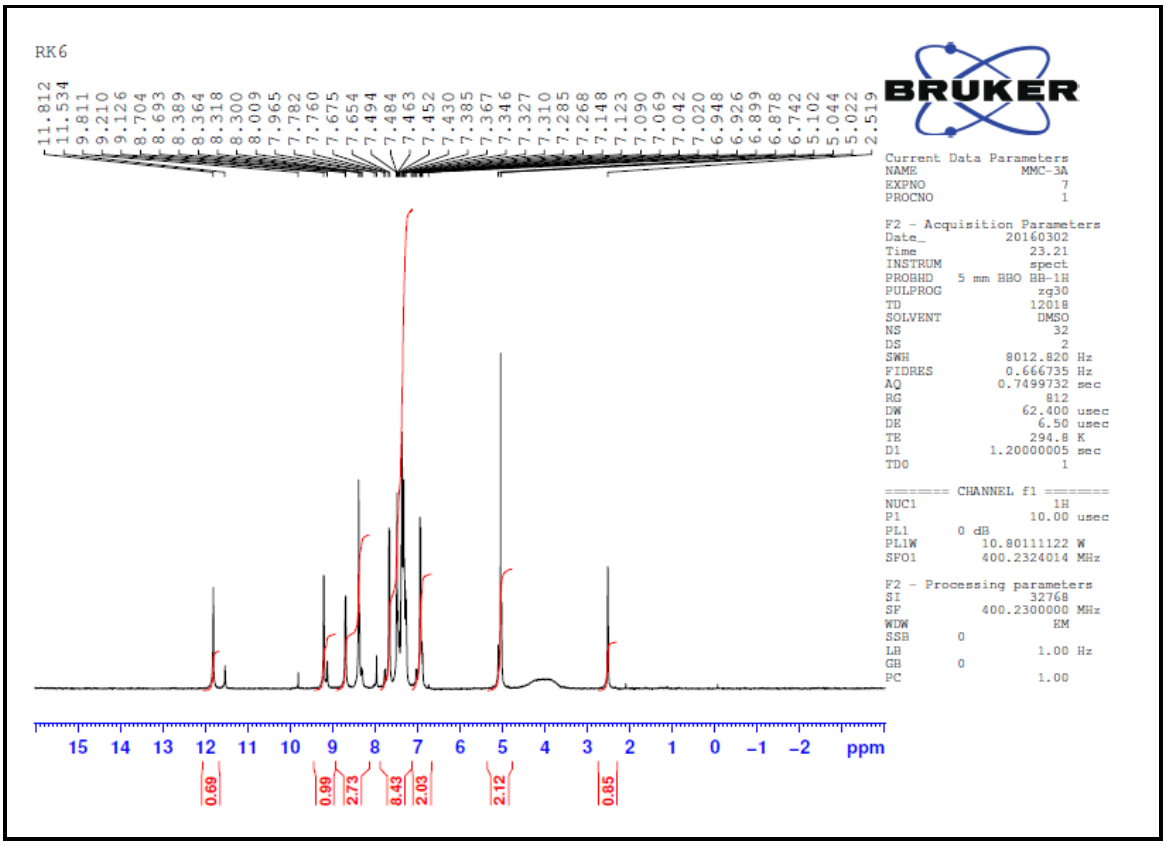




Actual Molecular Mass : 331.36 g/Mol

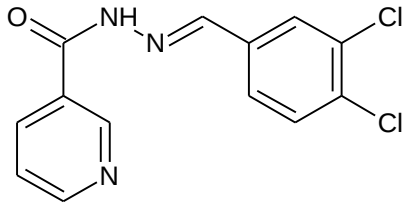
Expected Molecular Mass: 331.36 g/Mol

RK 6: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
1	Singlet	2.5ppm
2	Doublet	5.1ppm
13	Doublet	6.9-9.3ppm
1	Singlet	11.9ppm

8. N'-[(E)-(3,4-dichlorophenyl)methylidene]pyridine-3-carbohydrazide

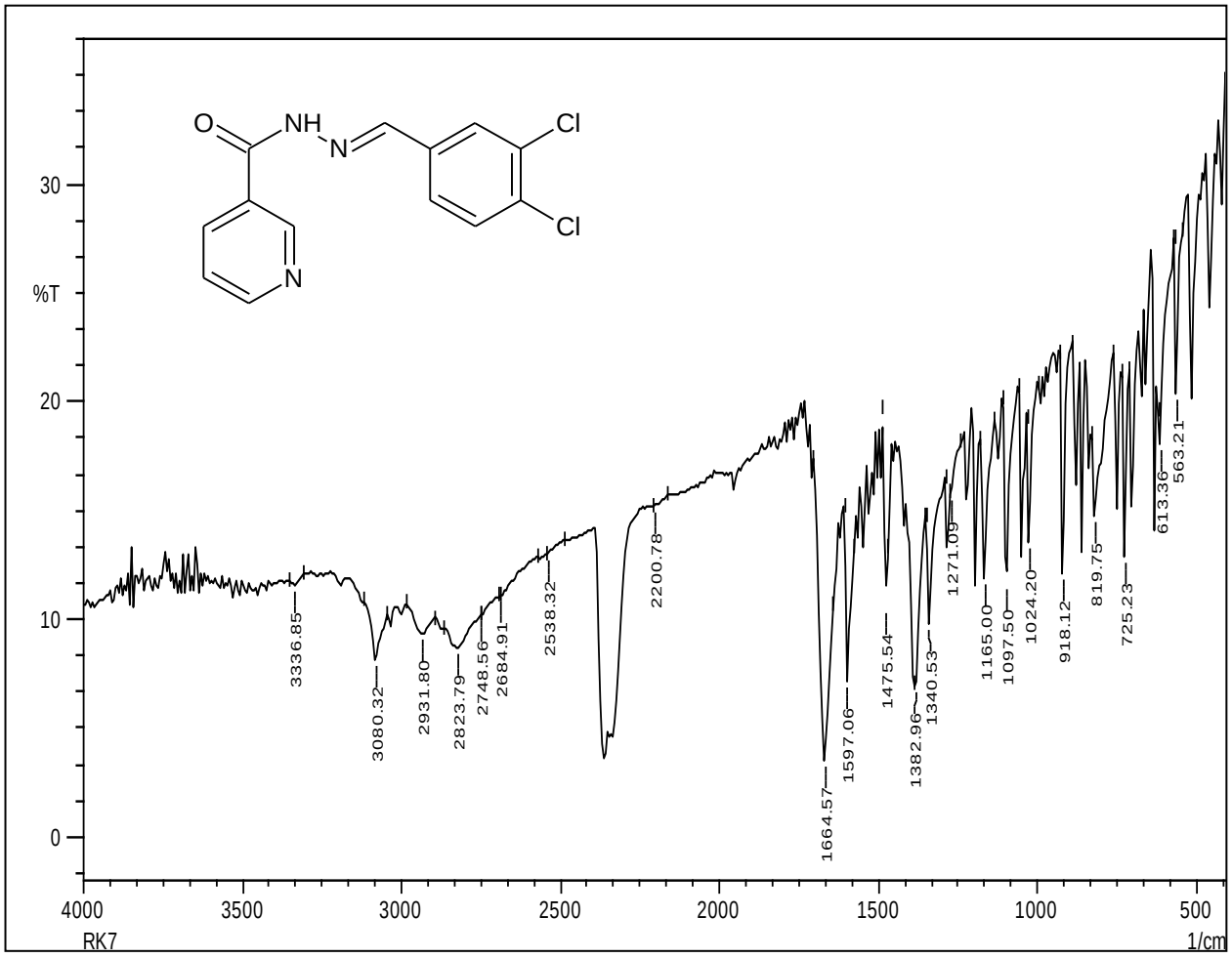


RK7

PHYSICO-CHEMICAL PROPERTIES

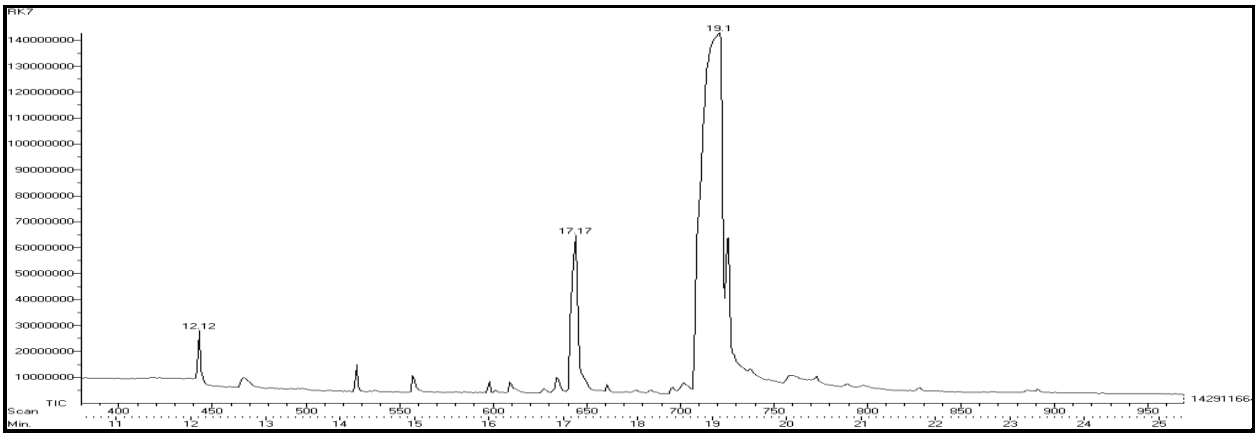
Description	:	Pale Yellow Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	136°C
Molecular Formula	:	C13H9Cl2N3O
Formula Weight	:	294.13606 g/Mol
Composition	:	C(53.08%) H(3.08%) Cl(24.11%) N(14.29%) O(5.44%)
Molar Refractivity	:	76.57 ± 0.5 cm3
Molar Volume	:	214.9± 7.0 cm3
Parachor	:	569.3± 8.0 cm3
Index of Refraction	:	1.631± 0.05
Surface Tension	:	49.2 ± 7.0 dyne/cm
Density	:	1.36 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	30.35± 0.5 10-24cm3
Monoisotopic Mass	:	293.012267 Da
Nominal Mass	:	293 Da
Average Mass	:	294.1361 Da
M+	:	293.011719 Da
M-	:	293.012816 Da
[M+H]+	:	294.019544 Da
[M+H]-	:	294.020641 Da
[M-H]+	:	292.003894 Da
[M-H]-	:	292.004991 Da

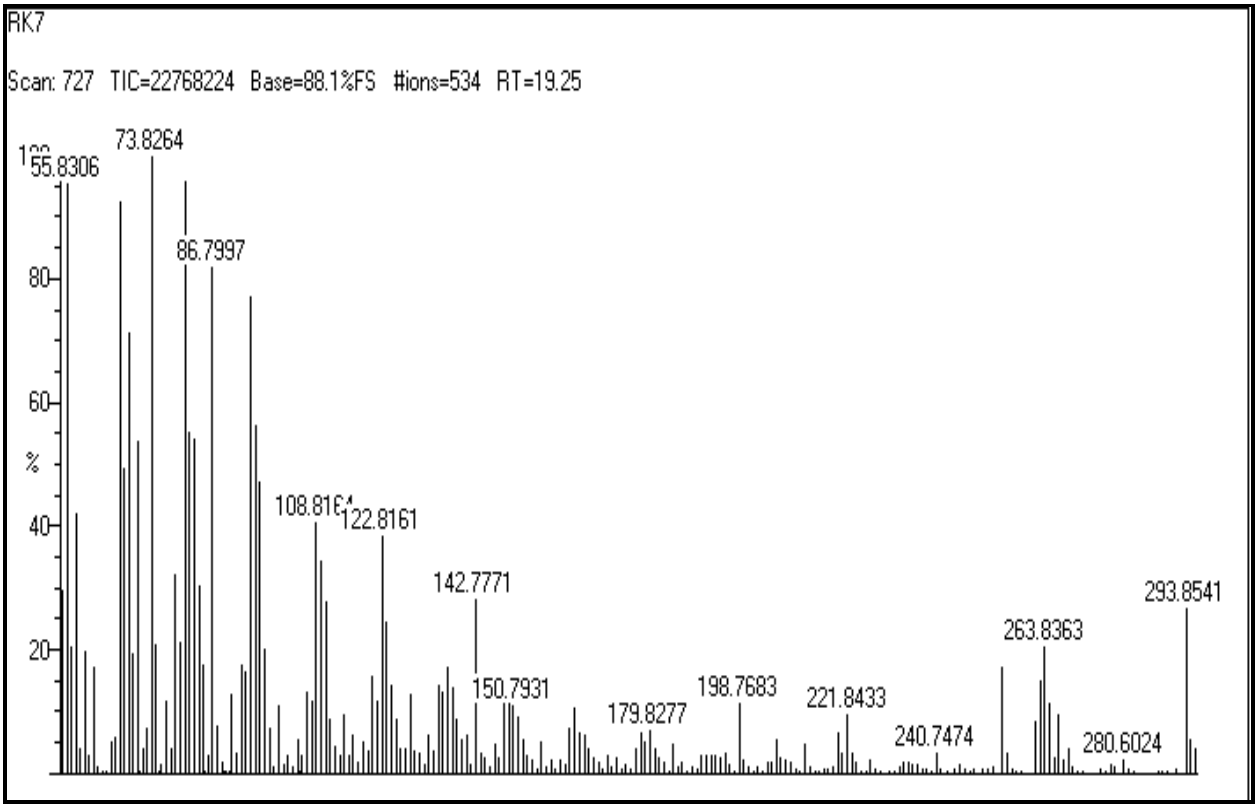
RK 7: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3080.32	C-H stretching	Aromatic
1664.57	C=O stretching	Amide
3336.85	N-H stretching	Amide
1597.06	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
725.23	C-Cl stretching	Halide group.
1597.23	C=C stretching	Aromatic

RK 7: GC – MS SPECTRUM

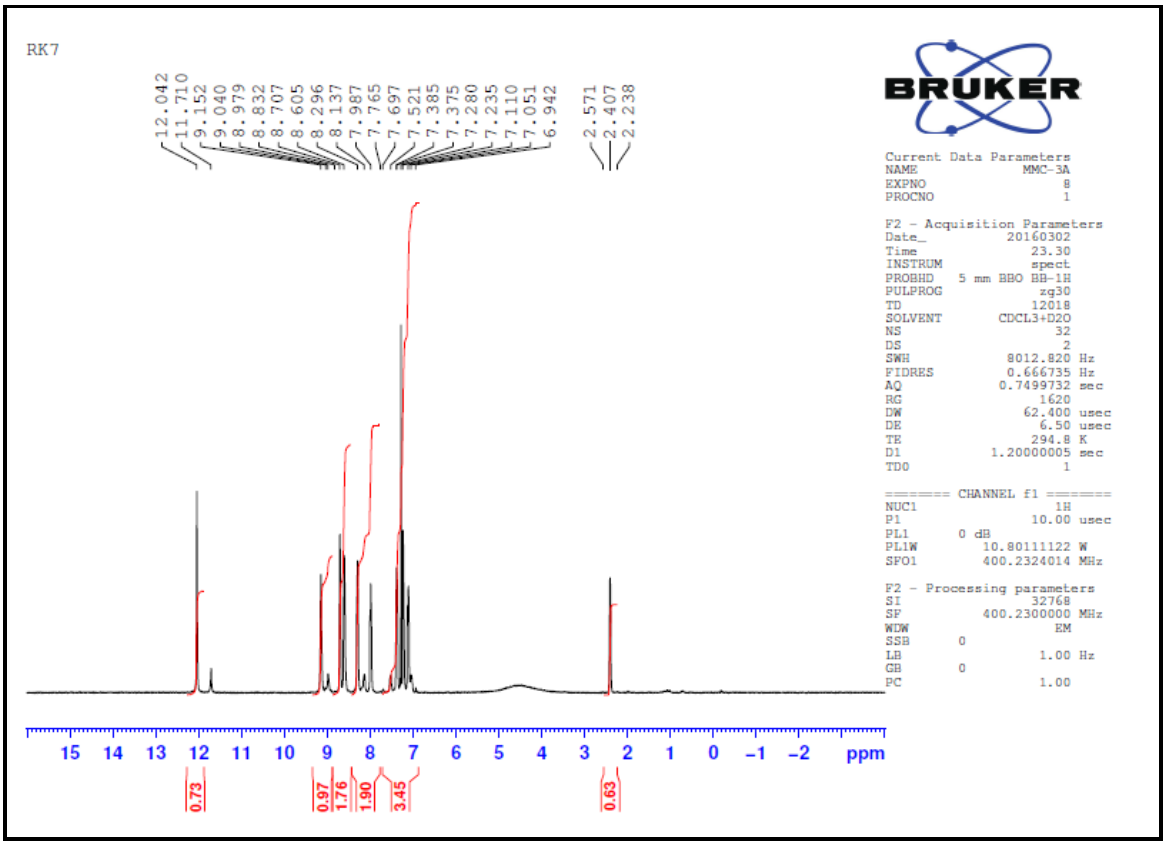




Actual Molecular Mass : 294.13 g/Mol

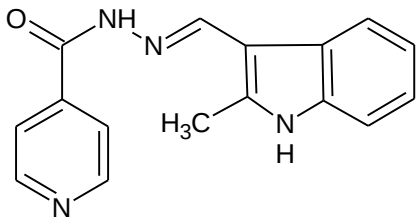
Expected Molecular Mass : 293.85 g/Mol

RK 7: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
1	Singlet	2.4ppm
3	Sextet	6.9-7.7ppm
4	Multiplet	7.8-9.2ppm
1	Singlet	12.1ppm

9. N'-[(E)-(2-methyl-1H-indol-3-yl)methylidene]pyridine-4-carbohydrazide

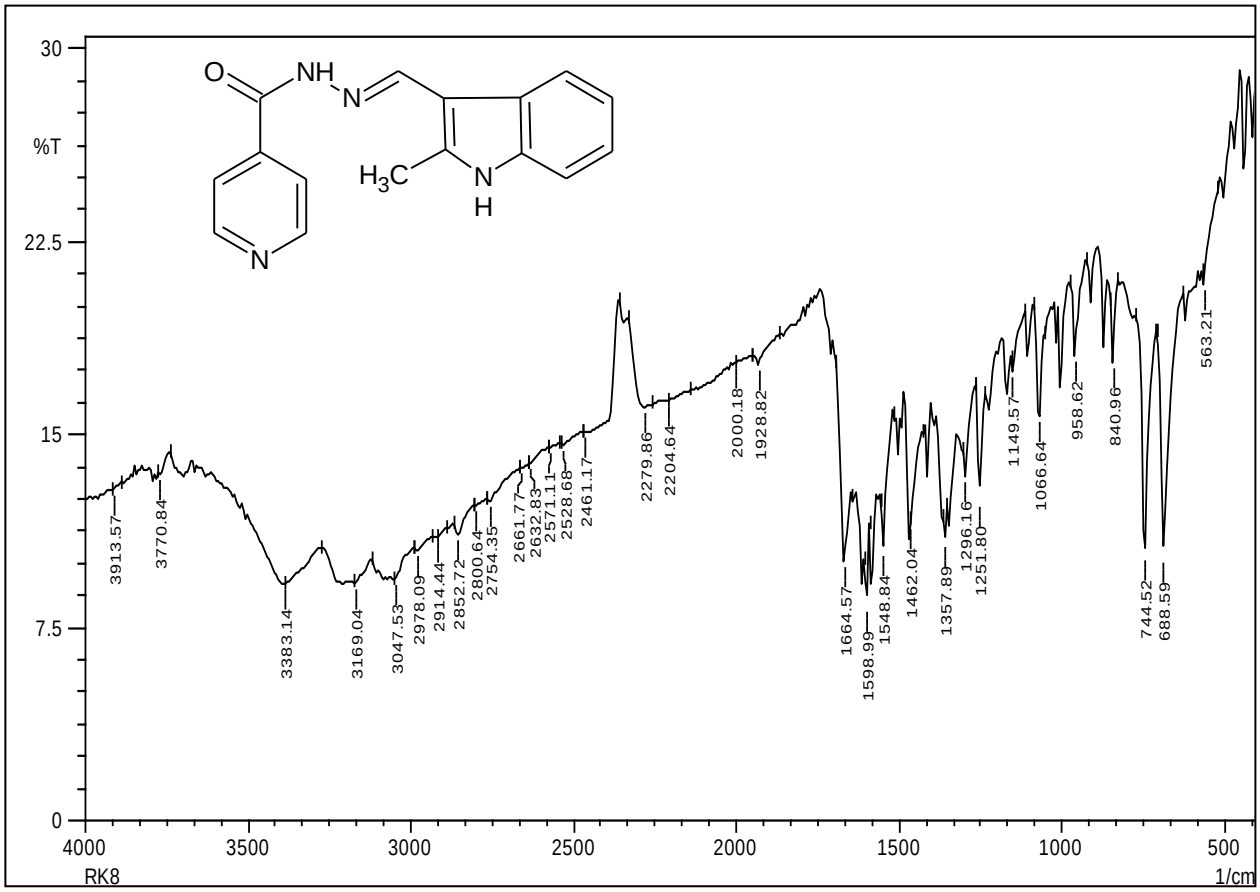


RK8

PHYSICO-CHEMICAL PROPERTIES

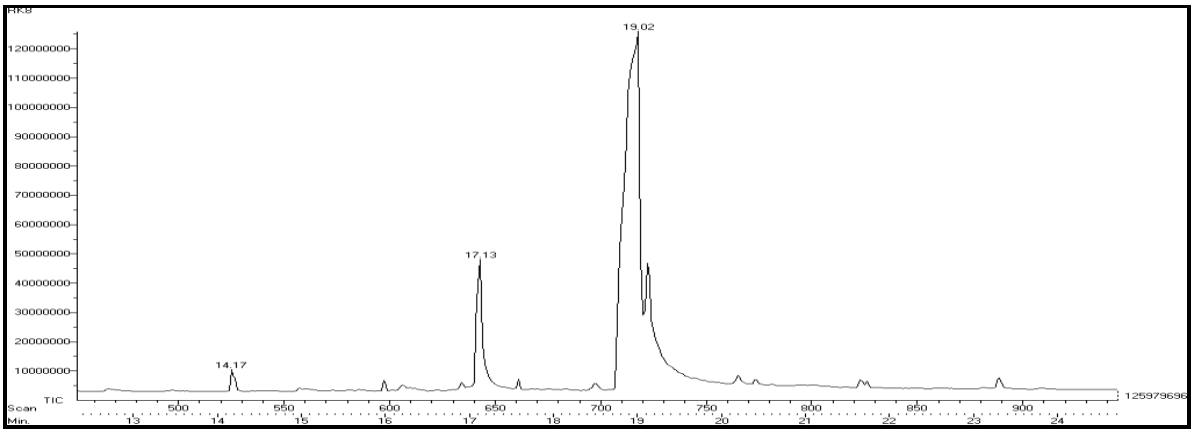
Description	:	Dark Yellow Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	242°C
Molecular Formula	:	C16H14N4O
Formula Weight	:	278.30856 g/Mol
Composition	:	C(69.05%) H(5.07%) N(20.13%) O(5.75%)
Molar Refractivity	:	81.51 ± 0.5 cm3
Molar Volume	:	219.9± 7.0 cm3
Parachor	:	584.9± 8.0 cm3
Index of Refraction	:	1.663± 0.05
Surface Tension	:	50.0 ± 7.0 dyne/cm
Density	:	1.26 ± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	32.31± 0.5 10-24cm3
Monoisotopic Mass	:	278.116761 Da
Nominal Mass	:	278 Da
Average Mass	:	278.3086 Da
M+	:	278.116212 Da
M-	:	278.11731 Da
[M+H]+	:	279.124038 Da
[M+H]-	:	279.125135 Da
[M-H]+	:	277.108387 Da
[M-H]-	:	277.109485 Da

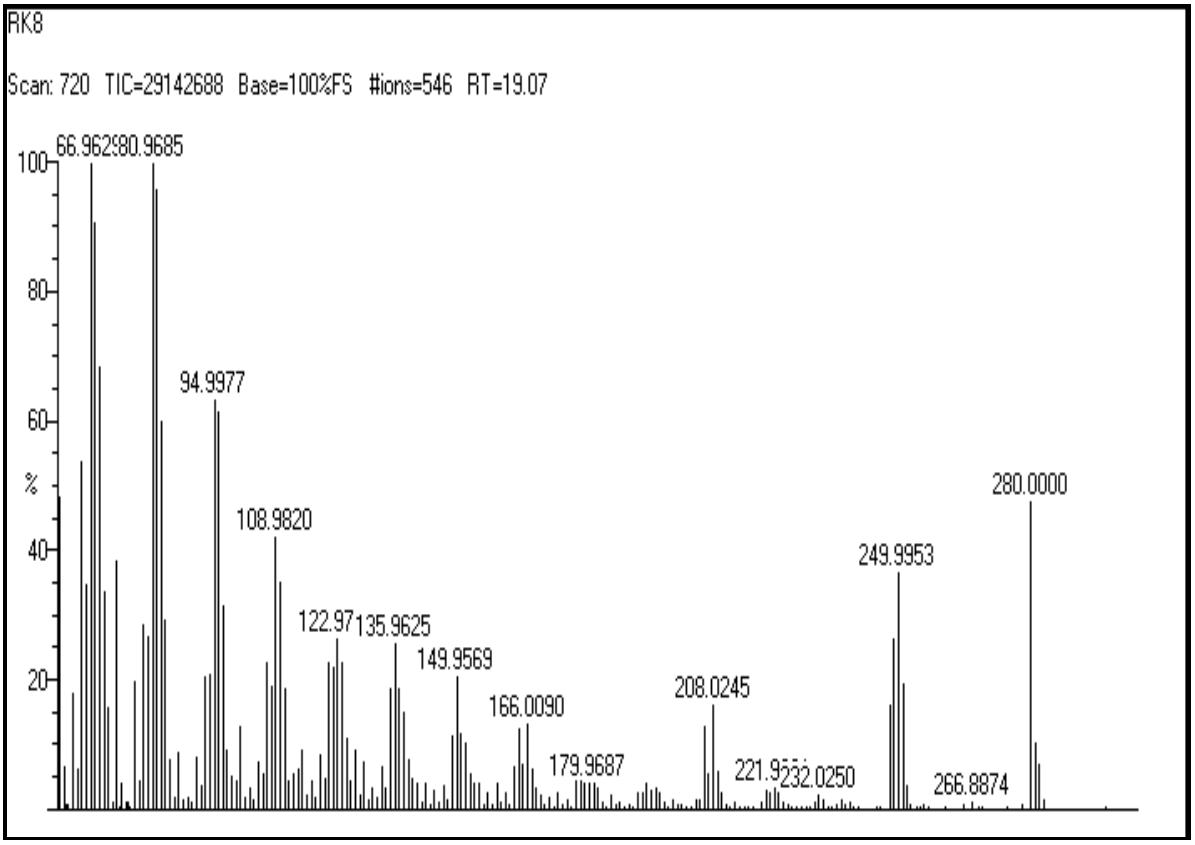
RK 8: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3047.53	C-H stretching	Aromatic
1664.57	C=O stretching	Amide
3383.14	N-H stretching	Amide
1598.99	C=N stretching	Aromatic (Ring in)
2279.86	C=N stretching	Aromatic (Ring out)
2852.72	C-H stretching in Methyl	Methyl group.
1548.84	C=C stretching	Aromatic

RK 8: GC – MS SPECTRUM

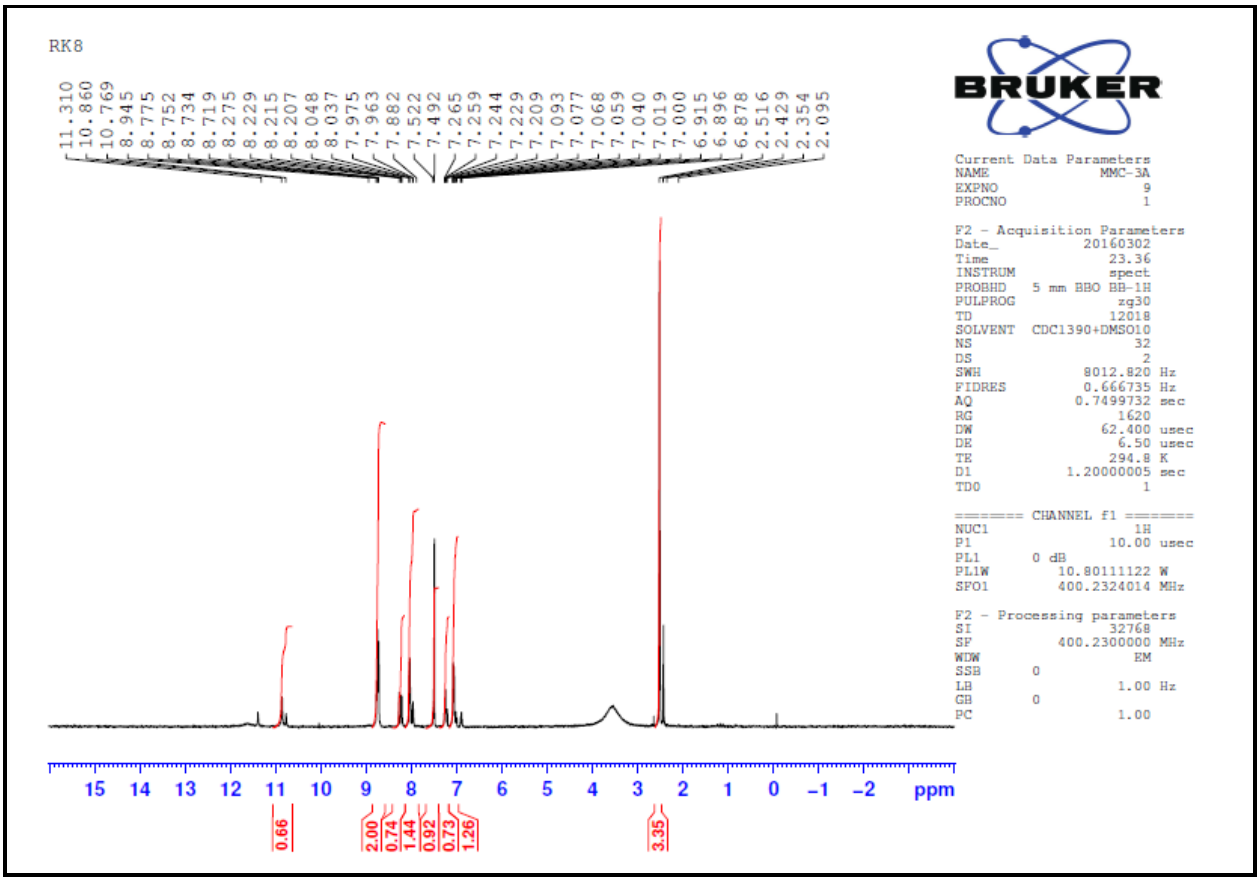




Actual Molecular Mass : 278.30 g/Mol

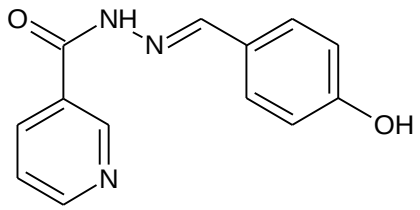
Expected Molecular Mass : 280.00 g/Mol

RK 8: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
3	Doublet	2.4-2.6ppm
9	Multiplet	6.8-8.8ppm
1	Doublet	10.8-10.9ppm

10. N'-[(E)-(4-hydroxyphenyl)methylidene]pyridine-3-carbohydrazide

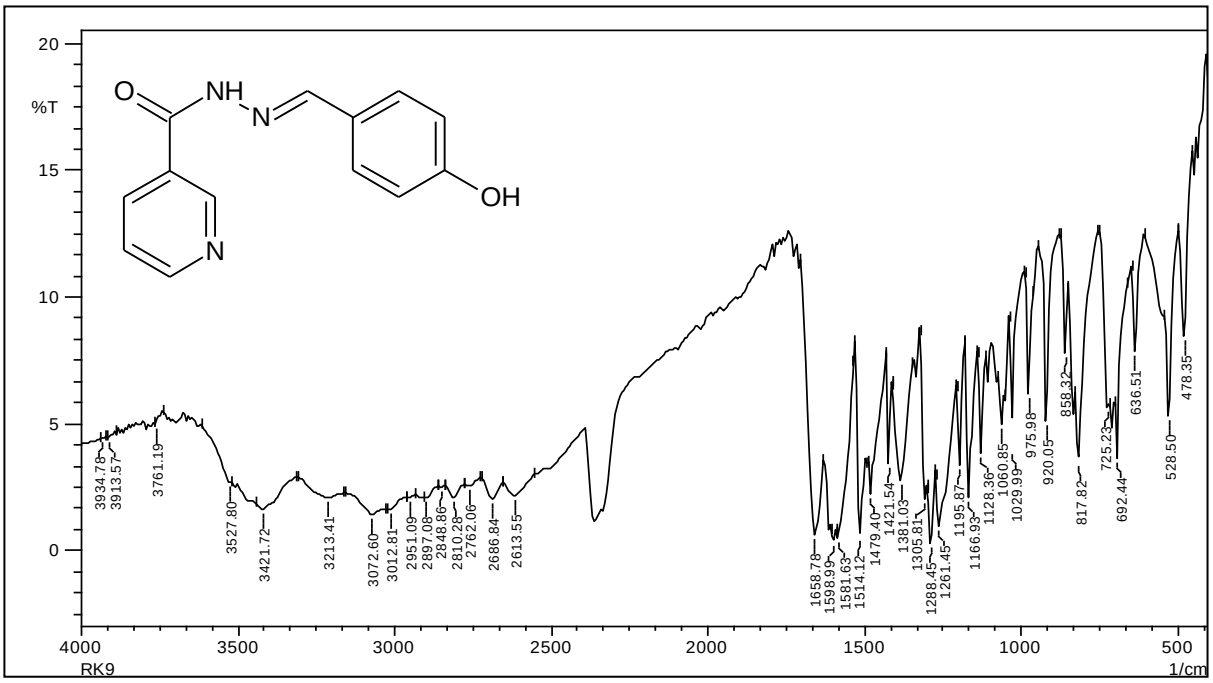


RK9

PHYSICO-CHEMICAL PROPERTIES

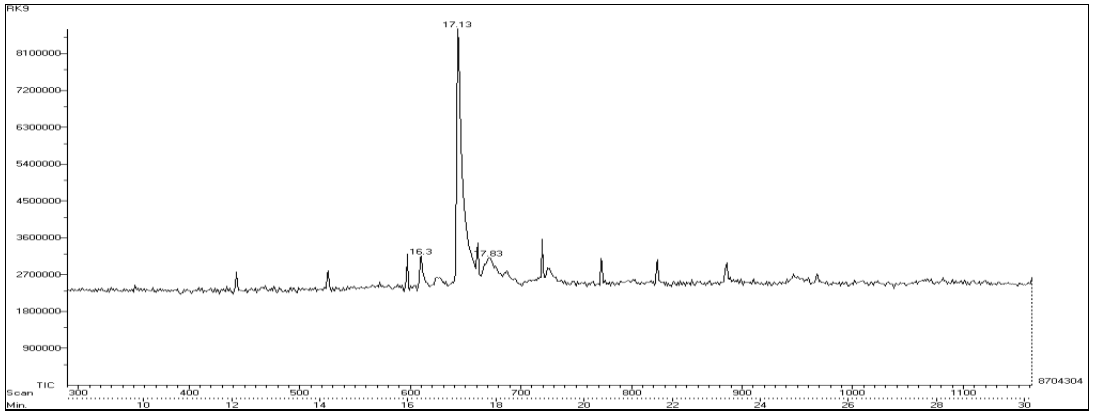
Description	:	Pale Yellow Crystals
Solubility	:	Insoluble in water, sparingly soluble in ethanol. Freely soluble in methanol, DMSO
Melting Point	:	215°C
Molecular Formula	:	C13H11N3O2
Formula Weight	:	241.24534 g/Mol
Composition	:	C(64.72%) H(4.60%) N(17.42%) O(13.26%)
Molar Refractivity	:	68.22 ± 0.5 cm3
Molar Volume	:	193.5± 7.0 cm3
Parachor	:	517.3± 8.0 cm3
Index of Refraction	:	1.622± 0.05
Surface Tension	:	50.9 ± 7.0 dyne/cm
Density	:	1.24± 0.1 g/cm3
Dielectric Constant	:	Not available
Polarizability	:	27.04± 0.5 10-24cm3
Monoisotopic Mass	:	241.085127 Da
Nominal Mass	:	241 Da
Average Mass	:	241.2453 Da
M+	:	241.084578 Da
M-	:	241.085675 Da
[M+H]+	:	242.092403 Da
[M+H]-	:	242.0935 Da
[M-H]+	:	240.076753 Da
[M-H]-	:	240.07785 Da

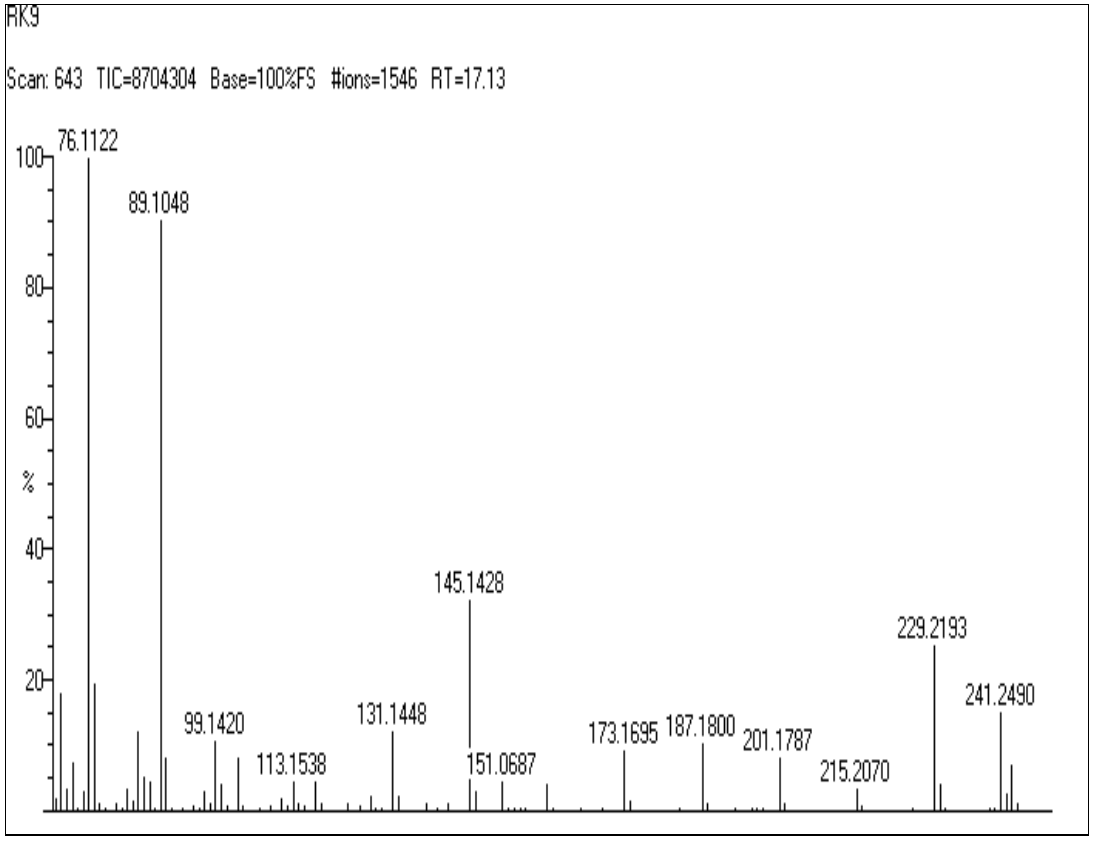
RK 9: IR SPECTRUM



Wave number cm-1	Functional group	Remarks
3072.60, 3012.81	C-H stretching	Aromatic
1658.78	C=O stretching	Amide
3421.72	N-H stretching	Amide
1598.99, 1581.63	C=N stretching	Aromatic (Ring in)
2360.87	C=N stretching	Aromatic (Ring out)
3527	O-H stretching	Hydroxy group.
1514.72	C=C stretching	Aromatic

RK 9: GC-MS SPECTRUM

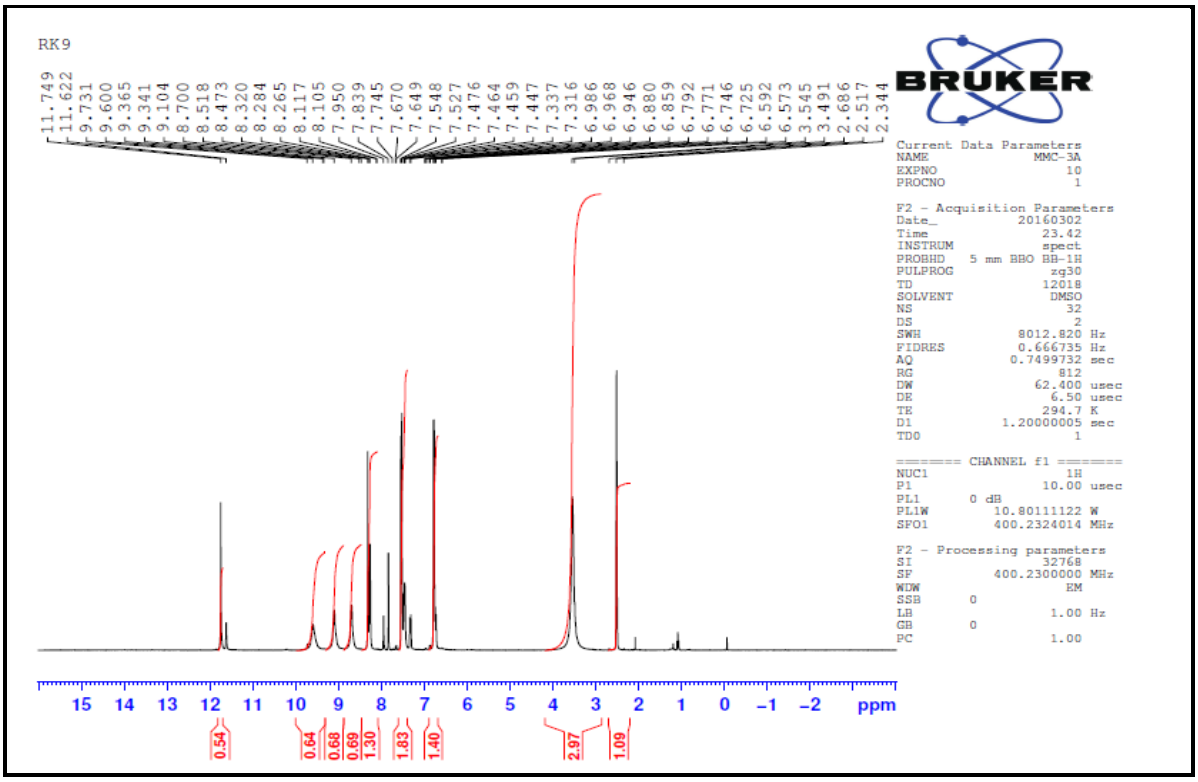




Actual Molecular Mass : 241.24 g/Mol

Expected Molecular Mass : 241.24g/Mol

RK 9: H^1 NMR SPECTRUM



NO OF PROTONS	TYPE OF PEAKS	δ VALUE
1	Singlet	2.5ppm
3	Singlet	3.55ppm
6	Multiplet	6.8-9.7ppm
1	Doublet	11.8ppm

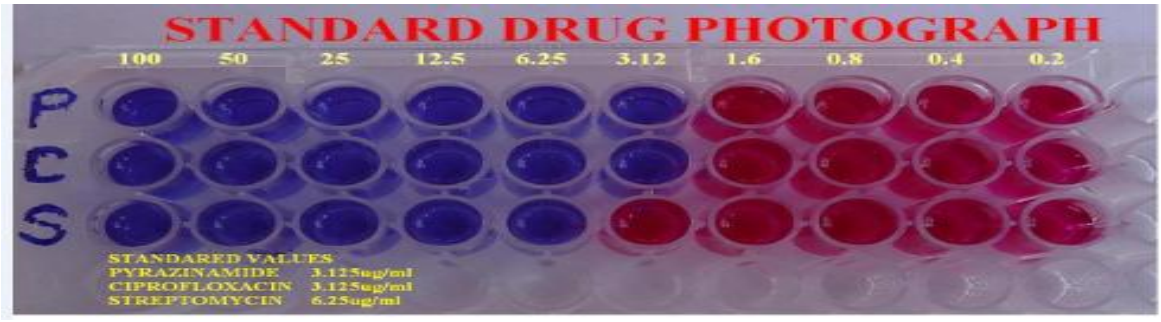
RESULTS OF BIOLOGICAL SCREENING

The synthesized compounds were screened for their in-vitro anti mycobacterial activity by means of alamar blue assay. The compounds were tested in the concentration range of 100 to 0.8 µg/ml against *M.tuberculosis* H37Rv strain grown in Middlebrook 7H9 broth in 96 well titre plate. Pyrazinamide-3.125µg/ml, Ciprofloxacin- 3.125µg/ml and Streptomycin- 6.25µg/ml were used as standards for comparison. A blue color in the well was interpreted as no bacterial growth so it is termed as sensitive, and pink color was scored as growth and is referred as resistant. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

MABA REPORT OF THE SYNTHESISED COMPOUNDS

Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	200 ng/ml
RK-1	S	S	S	S	S	R	R	R	R
RK-2	S	S	S	S	S	S	R	R	R
RK-2a	S	S	R	R	R	R	R	R	R
RK-3	S	S	R	R	R	R	R	R	R
RK-4	S	S	R	R	R	R	R	R	R
RK-5	S	S	R	R	R	R	R	R	R
RK-6	S	S	R	R	R	R	R	R	R
RK-7	S	S	R	R	R	R	R	R	R
RK-8	S	S	R	R	R	R	R	R	R
RK-9	S	S	R	R	R	R	R	R	R

STANDARD DRUG PHOTOGRAPH



SYNTHESISED COMPOUND PHOTO GRAPH

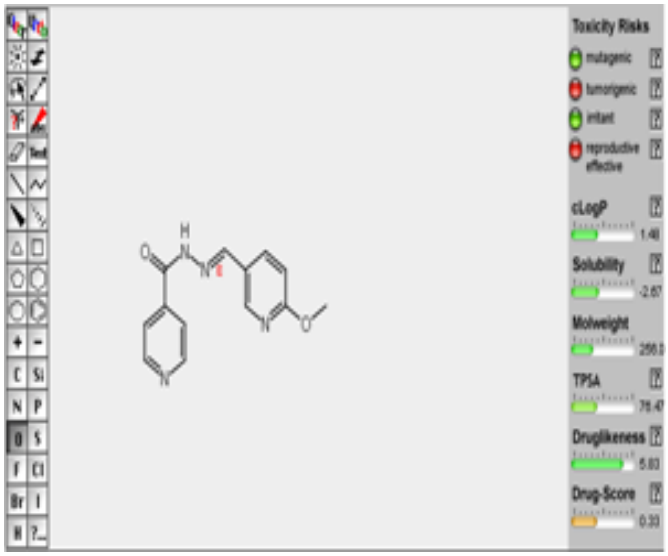
Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
RK-1								
RK-2								
RK-2a								
RK-3								
RK-4								
RK-5								
RK-6								
RK-7								
RK-8								
RK-9								

RESULTS OF TOXICITY RISK ASSESSMENT

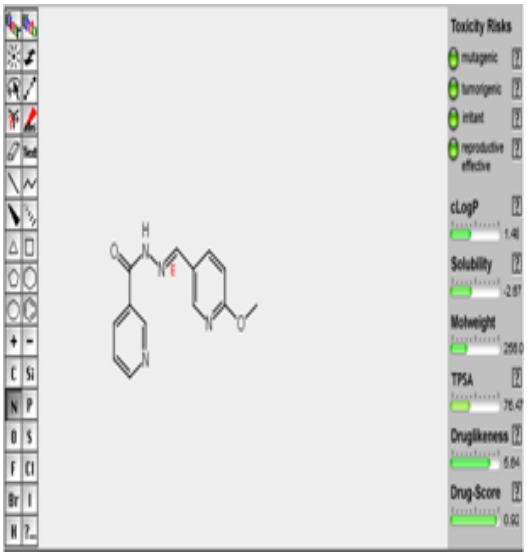
All the data set molecules were subjected to the toxicity risk assessment by using **OSIRIS property Explorer** . It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. The properties are cLogP, solubility, molecular weight,total polar surface area (TPSA), druglikeness and drug score.

INSILICO TOXICITY PREDICTION

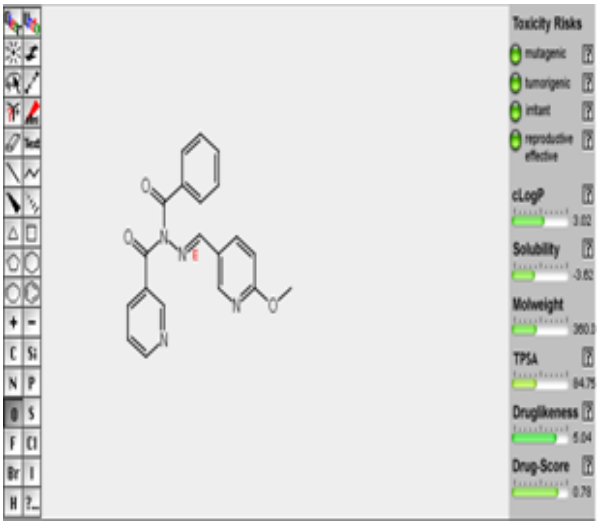
Prediction results are color coded in which the **red colour** shows **high** risks with undesired effects like **mutagenicity** and **reproductive effects** or a poor intestinal absorption and **yellow colour** shows **moderate** risks with undesired effects and **green colour** indicates **drug-conform** behavior.



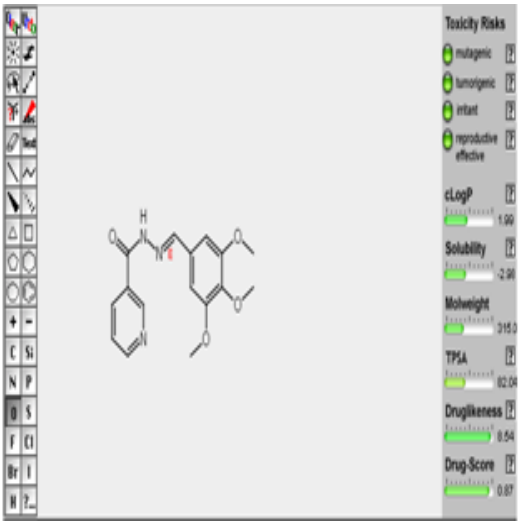
RK 1



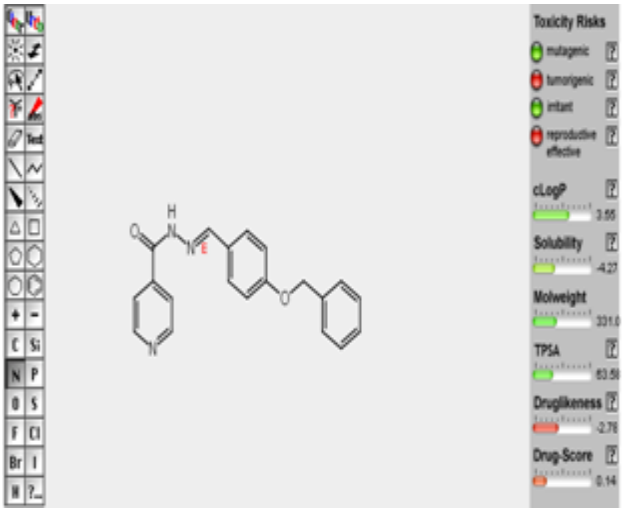
RK 2



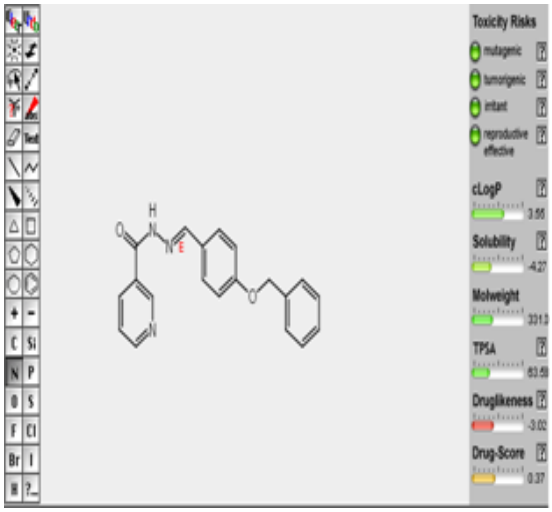
RK 2a



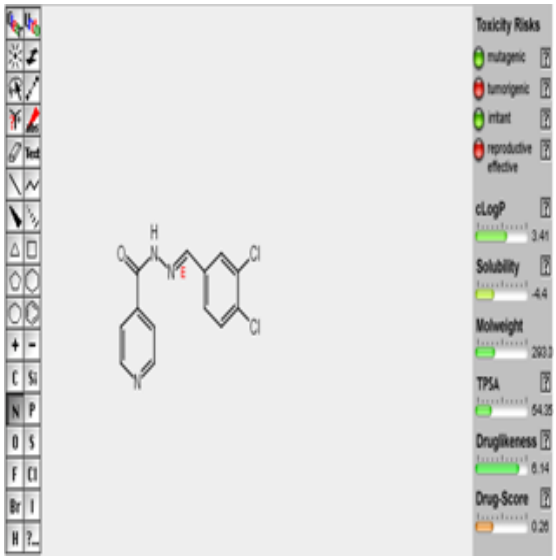
RK 3



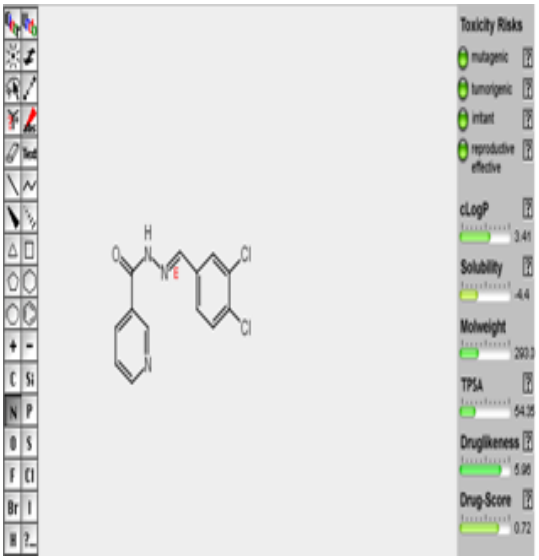
RK 4



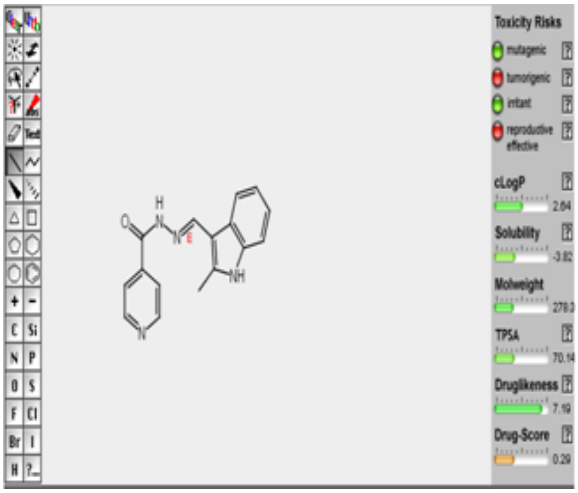
RK 5



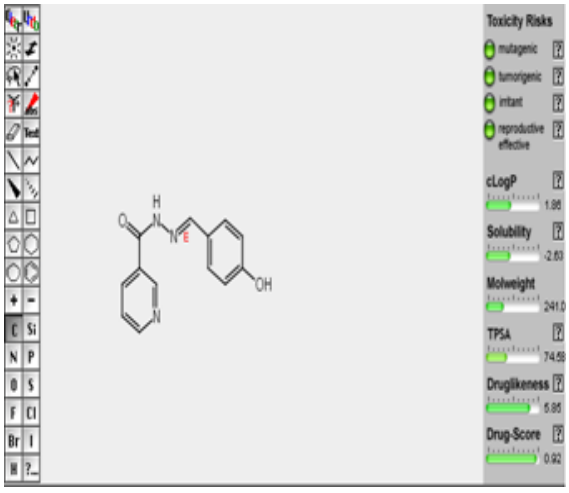
RK 6



RK 7



RK 8



RK 9

DISCUSSION

- The synthesised compound RK1and RK2a showed a 100% purity due to presence of single peak in LC-MS spectrum and GC-MS spectrum respectively, and its purity were confirmed by sharp melting point at 165°C and 88°C

- The synthesised compounds RK3, RK4, RK5, RK6, RK7, RK8 and RK9 showed above 90% purity due to presence of two or three peaks in spectrum , and its purity confirmed by sharp melting points.

- The invitro studies of synthesised compounds RK1 and RK2 were active at 6.25 µg/ml (MIC) and 3.125 µg/ml (MIC), which were compaed with the known standard drugs (MIC).

- The all compounds of RK2a, RK3, RK4, RK5, RK6, RK7, RK8, and RK9 were active at 50 µg/ml (MIC), which were not correlated with the any of the known standard drugs (MIC).

- Toxicity prediction of synthesised compounds were performed by Osiris property explorer and its toxicity characteristics were observed.

SYNTHESISED COMPOUNDS	MUTAGENIC	TUMAROGENIC	IRRITANT	REPRODUCTIVE EFFECTS
RK1	–	+	–	+
RK2	–	–	–	–

RK2a	–	–	–	–
RK3	–	–	–	–
RK4	–	–	–	–
RK5	–	–	–	–
RK6	–	+	–	+
RK7	–	–	–	–
RK8	–	+	–	+
RK9	–	–	–	–

NOTE: **(+) – TOXICITY** and **(–) NON-TOXICITY**

6. SUMMARY AND CONCLUSION

SUMMARY

Diaminopimelate decarboxylase (LysA), a critical enzyme for the lysine biosynthesis of Mycobacterium tuberculosis was chosen for study after review of literature.

Candidate molecules were designed and docked against 3C5Q protein using Argus lab 4.0software.

Molecules with good Docking score (lower binding energy) and interactions were shortlisted for synthesis. The reaction conditions were optimized.

The selected molecules were subjected to Toxicity prediction assessments by OSIRIS software. Only those molecules which did not indicate any form of toxicity were selected for synthesis.

Compounds were synthesized by conventional method and labeled as RK 1, RK 2, RK 2a, RK 3, RK 4, RK 5, RK 6, RK 7, RK 8 and RK 9.

Purity of the synthesized compounds was ensured by repeated recrystallization and purified by column and gas chromatography. Further the compounds were evaluated by TLC and Melting point determination.

The structures of the compounds were assigned on the basis of IR, NMR and MASS spectral data.

The pure compounds were screened for *IN-VITRO* Anti- tubercular activity by Micro plate Alamar Blue Assay (MABA). All compounds showed significant anti-mycobacterium activity.

The synthesized compounds were active at 3.125 – 50 µ g/ml, which is comparable to the known anti-TB drugs: MIC of Pyrazinamide - 3.125µg/ml, MIC of Ciprofloxacin - 3.125µg/ml and MIC of Streptomycin - 6.25µg/ml.

Though the synthesised molecules were designed for the target Diaminopimelate Decarboxylase (LysA), the molecules were docked against other critical anti-tubercular targets like Alpha 1,4-N- Acetyl glycosaminyl transferase(1,4NAGT), D-3 Phosphoglycerate dehydrogenase(D3PD), Pyridoxamine 5-phosphate oxidase(P5PO) and D-Alanyl D-Alanine carboxypeptidase(DADAC).

It is found that compounds RK1, RK2, RK2a, RK3, RK4, RK5, RK6, RK7, RK8 and RK9 show better G-Score for the targets Alpha 1,4-N-Acetyl glycosaminyl transferase,D-3 Phosphoglycerate dehydrogenase, Pyridoxamine 5-phosphate oxidase and D-Alanyl D-Alanine carboxypeptidase.

CONCLUSION

Our work concludes that based on docking G-Score our synthesized molecules were effective in inhibiting the target enzyme Diaminopimelate decarboxylase (LysA)Score, which were important for the lysine biosynthesis of Mycobacterium tuberculosis.

Through the invitro studies, it were proved that the synthesised compounds RK1 and RK2 showed the MIC of 6.25µg/ml and 3.125µg/ml which were equal to the known concentration of the standard drugs streptomycin 6.25µg/ml and pyrazinamide 3.125µg/ml as well as ciprofloxacin3.125µg/ml.

All other compounds except RK1 and RK2 were active at 50µg/ml which were not correlated with any concentration of the known standard drugs.

The molecules were also docked with the some other enzymes, Based on the good G-Score the synthesised molecules were effective in inhibiting the target enzymes like 1,4 NAGT, D-3-PD, P-5-PO and DADAC, which were important for respectively the Arginine biosynthesis for regulates cell wall and cell processes , 6-Serine biosynthesis for cell signaling, Pyridoxine synthesis for intermediatory metabolism and respiration and Peptidoglycan synthesis for cell wall and cell processes of Mycobacterium tuberculosis.

FUTURE SCOPE OF THE STUDY

This work may be further extended to the following areas

- Screening the synthesised compounds for its activity against various other multifunctional drug targets of M.tuberculosis based on the good G-Score and ligand interaction.
- Carrying out further modifications in the structure, in case, the compound does not show the predicted activity against the target.

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